BIOSORPTION, BIOACCUMULATION AND FOOD CHAIN TRANSFER OF ORGANIC CHEMICALS

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FOREWORD

Issues relevant to "Food Chain Accumulation" (of contaminants) were initially and formally introduced to the Ontario Ministry of the Environment at a workshop organized by the Great Lakes Section, Water Resources Branch in July 1987. The proceedings of this workshop can be found in the Ministry of the Environment report, entitled "Proceedings of the Workshop on Aquatic Food Chain Modelling", organized by Yousry Hamdy and Gary Johnson (MOE) and J.A. McCorquodale (University of Windsor), May 1988. Shortly thereafter, the Ontario Ministry of the Environment (by way of contract to an external consultant) addressed a number of questions raised at the workshop. Techniques to enhance the Water Resources Branch's ability to predict food chain accumulation of contaminants, within biota of the Great Lakes aquatic ecosystem, was a main goal of this study. The report provides a review of the "state-of-the-art" of knowledge pertinent to bioaccumulation of organic chemicals by aquatic organisms.

The following topics are discussed:

1. sorption of chemicals from the water column to abiotic and biotic matter (i.e. bioconcentration);
2. uptake of chemicals from food (i.e. biomagnification);
3. metabolism, excretion and transfer of contaminants through food chains to higher trophic levels.

It is of prime importance, when considering the fate and significance of "trace" contaminants within the aquatic ecosystem, that the concept and implications of bioconcentration of contaminants to aquatic organisms be carefully taken into account. This is particularly true for chemicals of low or moderate hydrophobicity. The report provides two (2) major conclusions with respect to this phenomenon. The first is that the process is thermodynamic in nature, i.e. biota absorb chemical to establish an appropriate equilibrium status with respect to the water. And, the second conclusion states that: "Bioconcentration is thus not caused by a difference in rates, the different rates are the result of an approach to equilibrium".

Biomagnification, i.e. the dietary bioaccumulation of organic chemicals by single organisms is significant for very hydrophobic chemicals. The biomagnification process is extended to the food chain level of complexity. The food chain process incorporates "sequential" biomagnification, as trophic levels are increased. This final phase of bioaccumulation is of major economic importance, due to chemical (contaminant) uptake by large sport fish.

The final three chapters of the report present a series of calculation procedures for bioconcentration and biomagnification and laboratory methods for the investigation of bioconcentration phenomena. A comprehensive bibliography of approximately 120 references, as well as an appendix of selected relevant physicochemical properties for priority chemicals and bioconcentration data are also reported.

The information provided in this report establishes a good data base, to assist the Ontario Ministry of the Environment in setting effluent limits based on receiving water quality. These effluent limits will, in turn, be compared with technology based effluent limits, derived through the MISA process. Such comparisons will greatly enhance the Ontario Ministry of the Environment's ability to recommend/evaluate load reductions achieved through the MISA program. And, as well, provide guidance and rationale for future policy decisions. Research and monitoring activities on the Great Lakes will, based on the information in this report, endeavour to incorporate design strategies, which more efficiently and reliably obtains data critical to those models addressing Food Chain Bioaccumulation dynamics.

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SUMMARY

This report has been prepared for the Ontario Ministry of the Environment to provide a comprehensive review of the state of knowledge of bioaccumulation of organic chemicals by aquatic organisms, especially fish. The topics which are discussed include sorption of chemical in the water column to abiotic and biotic matter, bioconcentration (i.e. uptake by the organism from water), uptake from food, metabolism, excretion and transfer through food chains to higher trophic levels.

Procedures are described for calculating the biotic concentrations arising from these processes at three levels of complexity. Illustrative calculations are provided.

Methods of measuring these processes in the laboratory are reviewed.

A comprehensive bibliography of some 120 references is provided. An appendix contains selected relevant physical-chemical property information for priority chemicals and reported bioconcentration data.

It is hoped that the report will serve to make available to Ministry staff the state of the art in this subject area, and enable them to undertake bioaccumulation estimations which are as accurate as permitted by our present knowledge of these phenomena.

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1. INTRODUCTION

1.1 Objectives, Scope and Definitions

The Ontario Ministry of the Environment is expanding its water quality assessment capabilities as part of the Municipal and Industrial Strategy for Abatement or MISA program. Part of these assessments involves the prediction of the extent to which organic chemicals in the water column will partition into fish by gill and food uptake and are transferred through the food-chain to higher trophic levels. This report addresses these issues, reviews existing assessment capabilities and recommends assessment procedures based on the current state of knowledge.

The objective of this report is to provide the ministry with

(i) an up-to-date review of biosorption, bioaccumulation and food-chain transfer of organic chemicals.
(ii) a description of simple procedures to estimate, i.e. calculate bioaccumulation and food-chain transfer, for new and existing chemicals.
(iii) an outline of procedures to determine, in the laboratory, the bioaccumulation and food-chain transfer potential of organic chemicals.

Further, a number of suggestions or recommendations are made for research studies in areas in which there is inadequate knowledge. A comprehensive bibliography is also provided and lists of physical chemical properties and bioconcentration data are given for priority chemicals.

The overall process of bioaccumulation which is treated in this report consists of a sequence of events or processes by which chemicals, present in the water column as a result of a discharge, migrate into the tissues of aquatic organisms. Fish are the usual organisms of interest, especially from the viewpoint of human consumption. The observed concentrations in fish are often several orders of magnitude, e.g. 50,000 times greater than those in the water. Consequently, consumption of 1 litre or 1 kg of fish (which may be done in five meals) can result in exposure to a quantity of chemical equivalent to that of drinking approximately 50,000 litres of water, which is approximately a lifetime's consumption. It is thus important to identify those chemicals subject to bioaccumulation, and the quantitative extent of the bioaccumulation, since it is suggested that water quality objectives will take into account this exposure route, rather than considerations relating to toxicity to aquatic organisms or even drinking water objectives. In turn, these water quality objectives will dictate tolerable discharge rates and thus the necessity for water treatment processes or pretreatment programs.

The report is structured as follows:-

Chapter 2 addresses the issue of sorption of the chemical from the water column on to, or into, biotic or abiotic matter (especially natural organic carbon) which may render the chemical less "bioavailable". This process is particularly important for "hydrophobic" chemicals of low water solubility or high tendency to partition into organic phases.

Chapter 3 explains the concept of bioconcentration. It discusses the thermodynamics of the process by which chemical is absorbed by the organism from water (and desorbed to water) through respiring surfaces. A fish is unable to discriminate between oxygen and hydrophobic organic chemicals. Thus, it inadvertently absorbs the chemical through the gills. This process is fairly well understood and it is most important for chemicals of low or moderate hydrophobicity.

Chapter 4 considers bioconcentration kinetics, i.e. the rate at which concentrations are established in an organism as a result of exposure to water. Kinetics are especially important for highly hydrophobic chemicals which exchange very slowly with water. This slow exchange may be advantageous because it "protects" the fish from rapid build up in concentration, but once established, high concentrations in fish are slow to clear or depurate.

There is a common misconception that chemicals such as DDT or PCBs bioconcentrate because they are taken up rapidly and cleared only slowly, i.e. there is a large or fast uptake rate constant and a small or slow clearance rate constant. The bioconcentration phenomenon is the result of the high ratio of these rate constants, i.e. uptake/clearance. In reality uptake and clearance are usually passive transport processes with the same inherent rates or resistances. The phenomenon of bioconcentration is thermodynamic in nature, i.e. the fish is absorbing chemical to establish an appropriate equilibrium status with respect to the water, just as a solvent extracts chemical from water. During the uptake process the rate of uptake naturally exceeds that of clearance because the fish is at a concentration below that in equilibrium with water. When equilibrium is reached, uptake and clearance rates become equal. Bioconcentration is thus not caused by a difference in rates, the different rates are the result of an approach to equilibrium. Chapters 3 and 4 seek to clarify and quantify these phenomena.

Chapter 5 discusses dietary (food) bioaccumulation or "biomagnification" of organic chemicals by single organisms. This process is particularly important for very hydrophobic chemicals, which are appreciably absorbed in the gut, though slowly cleared.

Chapter 6 extends the biomagnification process to include food chain or food web transfer. In this process there is sequential biomagnification as trophic levels are increased, with top predators showing the highest concentrations. This is clearly significant due to the economic importance of larger sport fish, which are high in the food chain. Again this process is most significant for highly hydrophobic chemicals.

Chapter 7 presents a series of calculation procedures by which the phenomena in Chapters 2 to 6 can be quantified. Illustrative calculations are provided. Three levels of calculation complexity are given, the selection being presumably a function of the detail required and the importance of the situation.

Chapter 8 reviews laboratory methods by which bioconcentration phenomena can be investigated.

A bibliography is provided along with an appendix which contains (i) an extensive compilation of octanol-water partition coefficient data for priority chemicals. This partition coefficient is considered the most convenient descriptor of hydrophobicity and (ii) reported bioconcentration data.

Finally, it is useful at the outset to define certain terms extensively used in this report. Differences exist in the usage of these terms among workers. This may lead to some confusion. The following definitions are used.

Bioconcentration:	The process by which organisms absorb chemicals from the water resulting in chemical concentrations in the organism exceeding those in the water.
Biomagnification:	The process by which organisms absorb chemicals by ingestion of food resulting in chemical concentrations in the organism exceeding those in the food.
Bioaccumulation:	The process by which organisms absorb chemicals from water, food and other sources resulting in chemical concentrations in the organism exceeding those in the water.
Metabolic transformation:	The process by which the parent compound is enzymatically converted by the organism.
Food-chain accumulation:	The process by which the consumption of one organism by another organism results in an increase of the chemical concentration in organisms with an increase in trophic level.
Sorption:	The process by which a solute (i.e. a chemical or sorbate) in the water phase binds or interacts with matter (the sorbent) in the water column. It may include both absorption and adsorption
Bioavailability:	The bioavailability of a chemical is the fraction of the total amount of chemical in a medium (e.g. water or food) that can be absorbed by organisms. It usually refers to the non-sorbed or freely dissolved chemical in the water phase.
Egestion:	The process by which an organism clears digested food residues, and associated chemical, in feces.

Excretion: The combined processes of loss of chemical by metabolism and egestion.

Elimination: The process by which chemical is cleared from the organism through respiring surfaces, e.g. gills.

Depuration: The process by which chemical is cleared from the organism. This process involves elimination to the water (via respiring surfaces), egestion, metabolic transformation and other processes.

2. SORPTION

2.1 Introduction

In any assessment of bioaccumulation, the first, and most fundamental step, is to determine the total concentration of chemical in water and how that concentration is broken down to the amounts or proportions which are truly dissolved, and those which are associated with other "matter" present in the water. The other "matter" in the water column may take several forms:

1) dissolved electrolytes or inorganic species
2) dissolved organic material, including a variety of substances such as other pollutants, alcohols, ketones, carboxylic acids, phenols, proteins, and fulvic and humic acids
3) suspended inorganic matter, including clays, carbonates, and silicates
4) suspended organic matter, including some humic acids, microorganisms, ligneous matter, litter from plants, and small planktonic organisms
5) emulsified oils and other organic materials
6) there may also be present a surface organic micro-layer or "slick" on the water surface which may become incorporated into a water sample

The chemical nature of these materials is often in considerable doubt, and discrimination by analytical methods is difficult. Certain parameters are relatively easy to measure, including the electrolyte composition, the total concentrations of inorganic materials (especially metals) and the total quantity of organic carbon (TOC). Discrimination between dissolved and suspended matter is normally done by filtration using sub-micron filters, eg., 0.45 μm. In other cases, centrifuging may be used, and in research studies, methods such as dialysis, selective absorption or head space analysis may be used. Filtration is, however, the most common method of providing an operational definition for "dissolved and "suspended". But, there is little doubt that much suspended organic matter is present with particle sizes of approximately 0.1 μm, which is operationally defined as dissolved, while in reality it is not in true solution. Such particles, although small, are still very large when compared to molecular dimensions of, typically, 0.2 nm or perhaps 1/1000th of the size of the smallest particles which can be filtered out of solution. A comprehensive review of sorption in aquatic systems has been presented by Karickhoff (1984) to which the reader is referred for more detail on many of these aspects.

2.2 Sorbing Organic Matter

Of particular relevance in the case of organic chemicals are humic and fulvic acids and humin. These compounds consist of a spectrum of organic material, mainly of plant origin, with variable aromatic and alkane character, which contain acidic and other functional groups. These materials vary considerably in molecular weight and may be present in dissolved or suspended form, the relative amounts being dependent on pH. The lower molecular weight materials, or fulvic acids, are generally soluble in all reasonable environmental pHs. Humic acids dissolve only at low pHs. They are therefore generally regarded as being in suspended form. They normally impart a brown colour to the water. Humin, a still higher molecular weight material, is totally in suspended form.

At the present state of chemical analytical capability, the best that can be done on a routine basis, is to determine the total organic carbon, and after sub-micron filtration, the dissolved organic carbon (DOC). As is discussed later, more sophisticated measurements can be made at the research stage.

The primary phases of sorptive capacity for organic contaminants are the organic carbon containing materials. These may be fulvic acids, humic acids, living and dead organisms, litter from vegetation, oils, or accumulations of surface organic material. A considerable literature exists on the relationship between the sorptive capacity of such materials and the organic carbon content. Karickhoff (1984) has conveniently reviewed this literature.

There remains some controversy about the state of the chemical when sorbed to the organic matter. The most generally held current belief is that the chemical is either dissolved in the lipid or lipid-like phases within the organism, or it is essentially present in a solid solution form in, or on, a sponge-like matrix of humic or fulvic material.

Perhaps the best available simple phenomenological explanation is that an organic molecule, such as a PCB, causes considerable distortion of the surrounding local water matrix. This forces the water to establish a structure which is energetically unfavourable. Any process which minimizes this distortion, by reducing the area between the organic molecule and the water, is highly favoured. An organic molecule can achieve this objective by dissolving in another phase, for example lipid or oil, or by associating with the surface of organic matter, thus displacing water from that surface. Such chemicals are generally referred to as "hydrophobic" or water hating. The primary task is to develop a method of calculating the extent to which this sorption occurs, as a function of readily available physicochemical properties of the chemical of interest, and of readily measurable properties of the sorbing material in the water.

2.3 Sorbing Mineral Matter

It appears that wet mineral surfaces play a relatively minor role in sorbing organic chemicals in comparison to organic matter. It should be noted that dry mineral surfaces may have a high sorptive capacity, but this is irrelevant to aquatic systems. It is possible that, as a result of microbial action, most mineral surfaces in the aquatic environment become covered with layers of living and dead microorganisms. Thus, the mineral surface is essentially shielded from the water by an organic layer.

2.4 Electrolytes

Dissolved electrolytes do not sorb organic molecules. Rather, they exert a salting out effect, thus increasing the activity or fugacity of the chemical. This can modify chemical behaviour considerably. There is a considerable literature on the effect of salt solutions on properties of dissolved organics, for example, Aquan-Yeun (1979). But most of this is relevant only to high concentrations in the range of 30 g/L, as occurs in salt water. Fresh waters in Ontario normally exhibit much lower salt concentrations. The effect of electrolytes can therefore usually be ignored. The exception would be discharges of chemicals into the waters of James Bay.

2.5 Surface Layers

We do not consider surface organic micro-layers further in this discussion. Their primary role is to act as a storage compartment for organic chemicals and possibly influence volatilization rates.

They probably play a relatively minor role in bioconcentration processes. Except, inasmuch as they provide higher concentrations at the surface, which may affect the food concentration of surface feeding fish.

2.6 Chemical Characteristics

Different organic chemicals display very different sorptive tendencies, largely because they vary in molecular size and in the presence of functional groups, such as hydroxyl or carbonyl groups. Shape also plays an important role. The most convenient characterization of this property of hydrophobicity is the octanol water partition coefficient, K_{OW}. The general concept is that assuming the lipid or organic carbon phase to be fairly similar in properties to octanol, then a chemical will partition between these phases and water with a ratio similar to that between octanol and water. This has proved to be a very reliable and useful working hypothesis.

Several correlations have been proposed between K_{OW} and lipid-water partition coefficients, K_{LW}, and organic carbon-water coefficients, K_{OC}. For lipid-water partitioning, Gobas et al. (1988) have recently shown that K_{OW} is approximately equal to K_{LW} for chemicals of log K_{OW} up to about 6. For higher K_{OW} values, the lipid water partition coefficient tends to be lower, possibly because of the structured nature of the lipid which resists deformation by the intruding sorbed molecules. For organic carbon, the primary recent work has been done by Karickhoff (1981), who has suggested that K_{OC} is proportional to 0.4 to 0.6 of K_{OW}. DiToro (1985) has recently suggested that, within experimental error, K_{OC} equals K_{OW}. Malcolm and MacCarthy (1986) and Gauthier et al. (1987) have suggested that this approach is somewhat naive and that the nature of the organic carbon, especially its aromatic content, varies greatly and should be taken into account. It seems likely that, in the future, techniques will be developed in which, not only is the organic carbon content measured, but its nature is characterized, possibly by the use of NMR techniques as suggested by Gauthier et al. (1987).

2.7 Calculating the Extent of Sorption

Where there exists a considerable concentration of organic carbon, for example in soils or sediments, excellent correlations are found between the bulk phase partition coefficients and K_{OW} using organic carbon content and K_{OC} as intermediaries. This is, perhaps, best illustrated by an example.

Example 2.1 We consider a sediment containing 5% organic carbon in contact with water containing a chemical with a K_{OW} of 10^5. K_{OC} may then be about 41000. The partition coefficient, K_P, is then 0.05 x 41000 or 2050. If the water concentration is 1 μg/L, then the bulk sediment concentration will be 2050 μg/kg. The assumption is that this bulk concentration consists of mixtures of 41000 μg/kg in the organic carbon phase and zero in the other mineral matter. In reality, this is probably not true, but as long as the mineral/water partition coefficient is less than about 200, the concentration on the mineral matter is less than about 200 μg/kg, and the amount associated with the mineral phase will not be significant (and will probably not be measurable) when in the presence of the much larger amount associated with the organic carbon. It can be noted that the concentration in the organic carbon phase of 41000 μg/kg is similar to the expected concentration in an octanol phase which would be in equilibrium with the water of 100000 μg/kg.

The first obvious approach is to apply this concept or calculation to suspended matter, as is illustrated in the following example.

Example 2.2 If a suspended matter is present at a total concentration of 20 mg/L and its organic content is 40%, then the concentration of organic carbon is 8 mg/L. If K_{OC} is 41000, then K_P becomes 16400. If the dissolved water concentration is 1 μg/L, the concentration in the organic carbon is 41000 μg/kg, and in the suspended sediments as a whole, it is 16400 μg/kg. But in each litre of water, there is 8 mg of organic carbon, ie., 8 x 10^{-6} kg, thus the amount of chemical associated with this material is 8 x 10^{-6} x 41000 μg, or 0.328 μg. Thus, the total concentration of chemical in the water is 1 μg dissolved plus 0.328 μg sorbed, ie., a total chemical analysis will show a concentration of 1.328 μg/L. 75% is dissolved and 25% is sorbed. Filtration should reduce this concentration to about 1 μg/L in filtrate, but in practice, it is more likely that the filtrate concentration will be about 1.1 μg/L because some of the chemical sorbed to organic carbon which will pass through the filter.

We can formalize this calculation procedure in the form of equations which are derived below using the following nomenclature:

Volume water (m^3) = V_W
Mass suspended sorbent (g) = M_S
Concentration of suspended sorbent = $m_S = M_S/V_S$ g/m^3 or mg/L
Fraction organic carbon = F (g/g)
Total amount of chemical (sorbate) = M (g)
Dissolved concentration of chemical = C_D g/m^3 water
Sorbed concentration of chemical = C_S g/m^3 water
Sorbed concentration of chemical = C_P g/g sorbent
Total concentration of chemical = C_T g/m^3 water
Partition coefficient = K_P L/kg or m^3/Mg

therefore

$$M = V_W C_T = V_W(C_D + C_S) \tag{2.1}$$

but

$$V_W C_S = M_S C_P \tag{2.2}$$

and

$$C_P = K_P C_D \times 10^{-6} \tag{2.3}$$

thus

$$m_S C_P = 10^{-6}\, m_S K_P C_D \tag{2.4}$$

and

$$C_T = C_D(1 + 10^{-6}\, m_S K_P) \tag{2.5}$$

$$C_D = C_T/(1 + 10^{-6}\, m_S K_P) \tag{2.6}$$

$$C_S = C_T \times 10^{-6}\, m_S K_P/(1 + 10^{-6}\, m_S K_P) \tag{2.7}$$

The key group is clearly $10^{-6}\, m_S K_P$, the magnitude of which controls the extent of sorption. Until recently, this approach has been widely accepted as valid, and has been used in mathematical models describing the fate of organic chemicals in aquatic systems. Indeed, in many situations, the approach is valid, but recent observations suggest that, under certain circumstances, the approach may be in substantial error.

2.8 Complicating Factors and Knowledge Gaps

The first issue is the question of reversible and resistant sorption. If experiments are conducted in which a chemical is adsorbed then desorbed in sequential steps, hysteresis is observed (DiToro and Hanzempa, 1982). It appears that some of the chemical becomes resistant to desorption. This is occasionally (and probably wrongly) termed "irreversible" sorption. It is probable that, given enough time, the material will desorb, thus the process is not inherently irreversible. The remaining chemical is labile or "reversibly" sorbed. The reasons for this difference are not entirely clear, but the current view is that the labile chemical is associated with the outer and more accessible surfaces of the sorbent. The resistant material may have migrated into the depths of this sponge-like phase and become trapped. DiToro (1985) has discussed this process in considerable detail and has developed equations and experimental techniques which can discriminate between these forms of sorption. Often the amounts in each phase are somewhat similar in magnitude. Most experiments have been done with soils, thus there is an element of doubt as to the applicability of these findings to suspended matter. This complication may not be a severe impediment to calculating acceptably accurate partitioning of the chemical in the water column, because we are primarily interested in the long term condition. It may, however, become important in situations in which the chemical in solution is exposed to rapidly changing sorbent concentrations.

For example, if a waste water contains a high suspended sediment concentration and that waste is rapidly diluted in a mixing zone, it is possible that the sorbed chemical will be slow to dissolve and adjust to the new suspended solids concentration. It may tend to remain associated with the solids and display an abnormally high apparent partition coefficient. Conversely, if in a water quality analysis, reliance is placed on the suspended matter in the receiving water to reduce the amount of dissolved chemical, i.e., reduce the availability of chemical for uptake in organisms from the water (i.e. its bioavailability), then it should be recognized that this sorption process may take some time. There may be a fairly rapid initial sorption, followed by a more prolonged final sorption. Further, exposure to filter feeding organisms may be variable during desorption and difficult to assess.

An area which has been very poorly studied and in which these processes become critically important is the resuspension of bottom sediments. If desorption is slow, then resuspended sediments, for example as generated by dredging, bioturbation, prop wash, storms or other disturbances, may result in sorbed chemical being conveyed into the water column. There is doubt as to the rate at which it will desorb prior to the suspended matter falling back to the bottom.

If natural organic matter has a content of resistant sorptive capacity, then that capacity may fill up fairly rapidly by various forms of dissolved organic material, including carbohydrates, proteins, and other natural products, as well as chemical pollutants.

The second, and more severe, problem is the "solids concentration" effect. O'Connor and Connolly (1980) reported that there was a trend for the partition coefficient, K_P, to fall as the sorbent concentration increased. This observation was based on results obtained in natural systems and was received with considerable skepticism. Many workers set out to demonstrate that the effect was in error and was simply the result of poor analysis, e.g. Gschwend and Wu (1985), Voice and Weber (1985). Carefully conducted laboratory experiments, e.g. Staples and Gerselman (1988), demonstrated that the effect is real and reproducible. It has been the subject of considerable discussion and controversy because it apparently contravenes the laws of thermodynamics, and no entirely satisfactory mechanism to explain it has been proposed. The effect is so important that we digress here to examine its nature and implications in more detail, again in the form of an example.

Example 2.3 We consider the previous example in which the solids concentration was 20 mg/L and K_P was 16400, the concentration of dissolved chemical in the water was 1 μg/L and the chemical concentration sorbed on to suspended material was 0.328 μg/L, totalling 1.328 μg/L. It is therefore 25% in sorbed form.

According to the equations presented earlier, if the suspended solids concentration were doubled to 40 mg/L, then more chemical would become associated with sorbent, the dissolved concentration will fall, the sorbed concentration will fall, but the total amount which is sorbed will rise, because there is a larger amount of sorbent. It can be shown that the same partition coefficient applies, the dissolved concentration C_D will drop to 0.8 mg/L, and C_S will rise to 0.53 mg/L. The percentage dissolved will therefore fall to 60%. In simple terms, if one doubles the sorbent concentration, one should approximately double the amount of chemical sorbed at least at low sorbent concentration ranges. In practice, what is typically observed is that at this new higher suspended solids concentration, K_P is reduced to perhaps 10000, and C_W does drop, but less than expected, to perhaps 0.95 μg/L. The percentage dissolved falls only to 71% instead of the expected 60%. In colloquial terms, it appears that the system is resisting the attempt to force more than half the dissolved chemical into sorbed form, by reducing its K_P. Thermodynamically, this is puzzling, because K_P should be independent of the sorbent concentration. How does the sorbent "know" what its concentration is?

> In some cases, it has been found that doubling the sorbent concentration causes K_p to become halved. Thus the addition of sorbent produces no net change in the dissolved water concentration.

These observations conflict so severely with established thermodynamic principles of partitioning that they have caused intense discussion and experimentation. The absence of a physical explanation of the phenomenon is particularly worrisome because, although empirical equations can be proposed to describe the effect, there remains doubt as to whether they can be reliably extrapolated to other chemicals, other sorbants, and other conditions. Only when there is an established mechanism, from which physical equations can be written representing that mechanism, can there be confidence that the effect is properly characterized.

Most workers in this area are divided into one of two viewpoints.

The first group, e.g. Gschwend and Wu (1985), Voice and Weber (1985) and Baker et al. (1986), are advocates of the "third phase" or "colloid" explanation. They point out that increasing the suspended solids concentration probably also causes an increase in the amount of sub-micron organic matter or colloidal matter in the water phase. Thus, the dissolved concentration may be considerably overestimated due to the presence of this material. There is no doubt that such colloidal material has a sorptive capacity and plays a considerable role in the process. The effect of colloids will be to increase the apparent dissolved concentration and thus reduce the overall partition coefficient. As the suspended matter concentration increases, so the colloidal concentration also increases, and K_p would be expected to decrease somewhat.

There are two problems with this explanation. First, if colloids do play a significant role in determining an apparent K_p, one would expect to find measured K_p values which are considerably lower than those estimated from the organic carbon partition coefficients. This is, in fact, not observed. Second, in order to obtain a decrease in K_p proportional to the increase in suspended solids concentration would require either that all the material in the dissolved phase is associated with colloidal matter and the amount of colloidal matter increases in proportion to the suspended solids concentration, or if not all the material is associated with the colloidal matter in solution, then the amount of colloidal material would have to increase more than in proportion to the solids concentration. Intuitively, these situations seem unlikely.

Perhaps the most convincing demonstrations that the colloid theory is, at best, only part of the explanation are the resuspension experiments, and the experiments using sorbents containing no organic carbon, conducted by DiToro (1985). There is little merit in presenting details of these experimental investigations here, but the evidence that they provide is convincing, at least to some, that the colloid effect is not the only process which is causing the anomaly.

The second group are advocates of the "collision" explanation. DiToro has presented an explanation in terms of two "reactions". The first "reaction" is sorption, in which an equilibrium is achieved between dissolved chemical and sorbed chemical, using a traditional partition coefficient or ratio of reaction rate constants approach. A second "reaction" is introduced, in which the sorbed material is subjected to desorption at a rate proportional to the product of the sorbed concentration and suspended particle concentration. This latter "reaction" is treated as being irreversible. Analyzing the steady state conditions leads to an equation (2.8) presented shortly, for the partition coefficient. It contains the ratio of adsorption to particle induced desorption rate constants, with a value of approximately 1.4 or 1/0.7. This model gives a good fit of data for a large body of adsorption/ desorption experiments. No molecular-based "reaction" explanation is offered.

Mackay and Powers (1987) have analyzed the same process and have postulated a somewhat different mechanism. They suggest that the reversible adsorption is rather a delicate process in which the sorbed molecules are attached to the outside of the organic particles only loosely. When two particles collide, the sorbed molecules are "shaken off" into solution. This implies that the energy involved in the collision is large compared to the energy of association or sorption between the sorbent and the solute. A particle of sorbent thus experiences a time history as follows. It migrates through the solution, picking up chemical and eventually approaching an equilibrium state. However, prior to reaching equilibrium, it suffers a collision with another particle, and the solute is shaken off. The particle then proceeds to pick up solute molecules again, and it thus undergoes a saw tooth history of sorbed concentration. At very low suspended solids concentration, the particles experience infrequent collisions. Thus, they spend most of their time at a state of equilibrium with the solution. In these conditions, there is no solids concentration effect. DiToro has observed this situation to apply when the product $10^{-6} K_P m_S$ is less than about 1, which implies that more than 50% of the chemical is in dissolved state.

As the suspended solids concentration increases, collisions become more frequent, and the suspended solids particles are unable to achieve an appreciable fraction of their equilibrium concentration. They are thus in a continual state of absorption and desorption. The mean apparent partition coefficient is far less than the equilibrium value. Increasing the suspended solids concentration increases the frequency of collisions per particle proportionately and thus reduces the concentration of the sorbed chemical, which explains the apparent drop in partition coefficient.

At first sight, it might appear that the partition coefficient should depend on the velocity of the suspended solids particle, but this is not the case. Increasing the velocity of the particle increases both the rate at which it encounters and sorbs chemical as well as its collision frequency. The net effect is for the velocity terms to cancel, and velocity has no effect on average amounts of sorption.

This collision explanation is still a hypothesis, and has been criticised by Lande (1988), and definitive experiments have not been conducted to prove or disprove it. Unfortunately, it is very difficult to probe the condition of the suspended solids-water mixture in a non-disturbing way. Filtration and centrifuging modify the nature of this solution and the velocity and collision rate of the suspended solids particles. Thus, they can never be used to investigate this phenomenon. The only feasible methods are to probe the condition of the dissolved material spectroscopically or by sensing the dissolved concentration indirectly in another phase, for example, by dialysis or head space analysis. It is expected that, in the next few years, experiments of these types will be conducted with a view to establishing the nature of the phenomenon.

For the present purposes, it is necessary to include characterization of this effect. It is recommended that DiToro's empirical model be used, since it gives a robust and accurate description of the observed phenomena. The recommended equation is as follows:

$$K_P = fK_{OC}/(1 + 0.7 \times 10^{-6}\ m_S f K_{OC}) \qquad (2.8)$$

where f is mass fraction organic carbon, K_{OC} is equated to K_{OW} and m_S is the solids concentration in units of mg/L or g/m^3, i.e. it is typically 1 to 10.

2.9 The Effect of "Dissolved" Organic Carbon

Several studies have been published recently addressing the issue of the extent to which dissolved organic species solubilize or increase the solubility of hydrophobic organic materials. Apparently, these organic co-solvents or solubilizing agents, increase the solubility of the chemical by modifying the water structure and making it more acceptable to the organic chemical. A consequence of this is that, at a specific concentration, they will reduce the activity or fugacity of the chemical, and thus probably reduce its bioavailability, except to filter-feeders. It is also essential that we quantify this effect. This topic has been investigated for a number of reasons. First, it is often desired to dissolve a sparingly soluble organic chemical in water by dissolving the chemical first in a solvent, such as ethanol or acetone, and then adding this mixture to the water. This avoids a problem of mixing a large volume of water with a small volume of sparingly soluble organic chemical, which could form a slick or become attached to the walls of the vessel. In pharmacy, this process is used to inject a quantity of a sparingly soluble drug. If the drug solubility is low, then the volume of water solution which must be injected to give the required dose of chemical may be too large to allow practical injection. To circumvent this, the drug is normally dissolved in a cosolvent, such as alcohol, in order to reach the desired concentration. It is important, however, that when this cosolvent-water-chemical mixture enters the blood stream, the chemical does not precipitate. There have been extensive investigations of the effectiveness of various cosolvents for various drugs.

Finally, this phenomenon is of interest to groundwater contamination. It is suspected that, in many cases, polar cosolvents, which may be formed from degradation processes in landfills, may increase the solubility of hydrophobic organic materials in water. This could reduce their sorption to aquifer materials, and thereby reduce the retardation factors, and increase their mobility.

The general finding from these studies, for example Morris et al. (1988), is that a plot of the logarithm of the solubility of the chemical (ordinate) versus the volume fraction of the cosolvent (abscissa) is generally linear, extending from the solubility of the pure solute in water on the left side to the solubility in the pure cosolvent on the right side. This relationship appears to be maintained for a number of solutes and a number of cosolvents.

If we invoke this theory to quantify the effect of dissolved organic matter in the water column, then we must first establish the solubility of the chemical in pure dissolved organic matter. This is an experimentally inaccessible quantity, but if we assume that the chemical's solubility in dissolved organic matter equals that in octanol, it is possible to estimate this value. The solubilities of most organic chemicals in octanol lie in the range 200-2000 mol/m^3. The solubility of the solute will thus increase as described by the following equation:

$$\log S = \log S_W + f(\log S_O - \log S_W) \tag{2.9}$$

where S is the solubility (mol/m^3 or g/m^3)
S_w is the solubility in pure water
S_o is the solubility in pure organic matter or octanol
f is the volume fraction of the organic carbon or cosolvent

The ratio S_o to S_w is approximately K_{oc} or K_{ow}, thus

$$\log S_o - \log S_w = \log K_{ow} = \log K_{oc} \tag{2.10}$$

and

$$\log (S/S_w) = f \log K_{ow} \tag{2.11}$$

The volume fraction organic carbon is usually in the range 10^{-5} to 10^{-7}, thus the cosolvent effect is slight unless K_{ow} is very large. For example, if f is 10^{-5}, ie., approximately 10 mg of organic carbon per litre, and log K_{oc} is 6, then log (S/S_w) is 6×10^{-5}, and solubility is enhanced from approximately 0.001 mol/m^3 to only 0.0010001 mol/m^3.

This increase is very small and would not be detectable. We thus conclude that, if the cosolvent equation (i.e. equation 2.11) applies, then under normal environmental conditions, the increase in solubility or decrease in activity attributable to truly dissolved organic carbon is negligible. Any solubilizing or activity reduction effect must be due to a second phase, i.e. particulate organic carbon.

The key question is "what fraction of the dissolved organic carbon is particulate and thus capable of sorption?" It is likely that no single answer can be given. The best that can be expected are typical ranges of values, specific to certain types of water bodies. Evans (1988) has recently used a dialysis technique to measure PCB sorption by DOC in a number of Ontario lakes and has found a mean value of approximately 2%. Other workers have found quite different values, for example Hassett (1988) for mirex in Lake Ontario found essentially 100%.

The best approach appears to be to use both 2% and 100% and test the sensitivity of the result to these assumptions. If an accurate value is required in a site specific case, measurements may be necessary. In general, this will only be needed when the product of K_{ow} and DOC (g/m^3) is equal or greater than approximately 10^5.

2.10 Recommended Calculation Procedure

From the foregoing discussion, we suggest the following procedure for calculating the extent of sorption in the water column for organic chemicals.

The total organic carbon content of the water is measured using conventional techniques. Water is then filtered through a sub-micron filter to remove the large particulate organic carbon. The total organic carbon content of the filtrate is measured again. By filtering the particulate organic carbon content can be determined. The organic carbon content filtrate is treated as consisting of two phases: dissolved organic carbon and colloidal organic carbon. A rigorous method of determining the amount of each is to measure the solubility of the hydrophobic organic chemical in this solution or to take a head space analysis or dialysis experiment. In the event that resources are not available to conduct these experiments, it can be assumed, based on the work of Evans (1988) that the material is (i) 2% colloid, 98% dissolved and (ii) 100% colloid. It is expected that, as data bases are created for various water bodies in Ontario, this dissolved value will become more accurately established. The particulate organic carbon and colloidal organic carbon contents are then combined and designated as the sorbing organic carbon content (SOC).

The next step is to establish K_{OC}. Following Karikhoff, this would involve multiplying K_{OW} by a factor of approximately 0.4 to 0.6, and following DiToro, it would involve a 1 to 1 conversion. Since, DiToro's measurements are more comprehensive and more recent, we prefer the factor of 1.0. An equilibrium partition coefficient, K_P, is then estimated from the suspended solids concentration m_S described earlier in equation 2.8. The calculation sequence is thus as follows:

Example 2.4 A PCB of log K_{OW} 6.5 is present at a total concentration of 5 ng/L (5 $\mu g/m^3$) in water. The total organic carbon content is 10 mg/L (10 g/m^3) of which 6 mg/L is particulate and can be removed by filtration and 4 mg/L is dissolved while the total suspended sediment concentration is 20 mg/L.

The sorbing organic carbon content is then either

(i) $6 + (0.02 \times 4) = 6.08$ mg/L, i.e. f is 6.08/20 or 0.20
or
(ii) $6 + (1.0 \times 4) = 10$ mg/L, i.e. f is 10/20 or 0.50

Equating K_{OC} to K_{OW} gives K_{OC} of $10^{6.5}$ or 3.10×10^6. K_P can thus be calculated from

$$K_P = fK_{OC}/(1 + 0.7 \times 10^{-6} m_S f K_{OC}) \qquad (2.12)$$

with m_S of 20, f of 0.3 or 0.5 gives

(i) $K_P = 66500$, or (ii) $K_P = 68400$

the fractions dissolved or bioavailable solute fractions (ASF) are

(i) $\text{ASF} = 1/(1 + 66500 \times 10 \times 10^{-6}) = 0.42$

or

(ii) $\text{ASF} = 1/(1 + 68400 \times 20 \times 10^{-6}) = 0.42$

The available concentration is thus 5 x 0.42 or 2.1 ng/L or approximately half the total concentration. The importance of the solids concentration effect is clear in that it has increased the bioavailable fraction from 3 or 5% to 42%.

A simple "rule of thumb" is that if $m_S f K_{OW}$ is less than 10^5, the solids concentration effect can be ignored and the available fraction will exceed 50%. As $m_S f K_{OW}$ increases to 10^6 and beyond the available fraction will fall to about 40% and will remain there regardless of the sorbent concentration, organic carbon content or the assumed values of 2 or 100% sorbing organic carbon.

The properties of the chemical are included in the calculation entirely in the form of the octanol water partition coefficient.

In conclusion, it must be emphasized that, although this method is believed to be valid and to yield reasonable results, not all the steps in it have been fully validated. There remains doubt about the nature and extent of the solids concentration effect, and about the proportions of colloidal dissolved organic carbon. It is hoped that, as more data become available for Ontario waters, these calculation procedures can be more fully validated, and if necessary, modified.

3. BIOCONCENTRATION

3.1 Introduction

Aquatic organisms are exposed to contaminants or chemicals in their environment by a variety of exposure routes. These exposure routes involve the direct uptake of chemicals from the surrounding water via the gills, the consumption of food, ingestion of sediments, filtration of suspended particles (e.g. by filter-feeders such as mussels), direct surface absorption and air-breathing (e.g. air-breathing fish). The relative importance of each of these exposure routes, as a source of contaminant uptake, differs from species to species and even within a species, depending on the physiological characteristics and requirements of the organisms, the nature and properties of the chemical and the ambient environmental conditions.

Although different species may have different routes of exposure, most aquatic organisms are in direct contact with the surrounding water, from which they extract oxygen. Aquatic organisms have developed more or less sophisticated methods to absorb oxygen from the water. In Oligochaetes oxygen uptake is predominantly a matter of diffusion. Fish, however, have developed gills for a more efficient uptake of oxygen. In the process of extracting oxygen from the water, aquatic organisms can also absorb other compounds present in the water. Especially when the substance in the water has a high affinity for the fish tissue relative to that for water, absorption of chemical from the water can be a very efficient process. This is the case for many organic chemicals such as aliphatic and aromatic hydrocarbons. These compounds have fairly low water solubilities and relatively high solubilities in lipids. This process of direct chemical transfer from water to the organism can result in chemical concentrations in the organisms which exceed those in the water. This is generally referred to as "bioconcentration", first reported by Hamelink et al. (1971).

Bioconcentration is a form of the more general process of "bioaccumulation". Bioaccumulation has been defined as the process by which organisms absorb chemical from water, food and other sources by various uptake routes (e.g. via the gills and gastro-intestinal tract). This results in concentrations in organisms exceeding those in the water or food. Bioaccumulation therefore involves chemical uptake from various sources (e.g. water, food and ingested sediments). Bioconcentration, however, refers only to chemical accumulation from the water. In this section the bioconcentration process will be discussed.

3.2 Bioconcentration: Mechanisms and Models

Based on studies with chlorinated hydrocarbons in laboratory ecosystems, Hamelink et al. (1971) proposed that chemical exchange between water and organism was the cause for elevated levels of these chemicals in their test organisms. This concept was quickly adopted and translated in a two compartment (i.e. fish and water) model with first order rate constants (Neely 1979, Branson et al. 1975). This model has been shown to give a satisfactory description of the bioconcentration kinetics for a variety of organic chemicals (such as halogenated aromatic and aliphatic hydrocarbons, polynuclear aromatics and aromatic and aliphatic alcohols) in many aquatic species. Various authors have shown that the mechanism of the bioconcentration process is basically a thermodynamically controlled partition process of the chemical between the lipids of the organism and the water (Konemann and Van Leeuwen 1980, Bruggeman et al. 1982 and 1984, Opperhuizen et al. 1985, Gobas et al. 1989). Excellent correlations between the bioconcentration factors and the 1-octanol-water partition coefficient for a variety of organic chemicals support this partitioning mechanism (Neely 1979, Veith et al. 1979, Mackay 1982). This conveniently allows the organism to be viewed as a "droplet" of lipid, and bioconcentration can be treated as a simple partitioning process of the chemical between this "droplet" of lipid and water.

Kinetic studies have shown that the bioconcentration process is generally well described by a two compartment (i.e. water and organism) model and that the kinetics of chemical bioconcentration follow first order kinetics (Konemann and Van Leeuwen 1980, Bruggeman et al. 1982 and 1984, Opperhuizen et al. 1985, Gobas et al. 1989). Studies demonstrating active (i.e. enzyme mediated) uptake of organic chemicals in aquatic organism have never been reported. It is possible that active transport may occur in biological membranes. Several ions and molecules with specific cellular functions are known to be actively transported through biological membranes. However, extensive studies on membrane permeation of simple organic chemicals have not shown any evidence of active membrane permeation in organisms (Stein 1986). The lack of existing active transport mechanisms for organic chemicals in organisms may be explained by the fact that, unlike ionic substances, many organic chemicals are non-polar and have a relatively high solubility in the lipids of membranes. This enables them to easily pass biological membranes which makes active transport redundant.

In this section we will discuss the organism-water model. This model has been validated by many studies and is generally believed to give an accurate description of chemical exchange between organisms and water. As a result it is often used for predictive purposes.

Organism-Water Two Compartment Kinetic Model

The simplest kinetic model describing chemical exchange between water and the organism is the organism-water two compartment model with first order kinetics. It can be expressed by the following reversible reaction model

$$C_W \underset{k_2}{\overset{k_1}{\rightleftarrows}} C_F \tag{3.1}$$

where C_W is the concentration of the chemical in the water, C_F is the chemical concentration in the organism, k_1 is the rate constant for chemical uptake from the water and k_2 is the rate constant for chemical elimination to or depuration from the fish back to the water. Typically, the units of C_F are in grams of chemical per gram of wet or dry weight of the organism. SI units of moles per litre or per cubic metre can also be used. The units of C_W have to be consistent with the units of C_F and are therefore often expressed as gram of solute per gram of water or as moles per litre. Typical units of the uptake rate constant k_1 are ml water per gram of fish per day e.g. ml water/g fish/d. However, it is frequently assumed that the density of the organism is equal to that of the water, i.e. 1.0 gram of organism per ml of organisms, i.e. 1.0 g/ml. This simplifies the units of k_1 to reciprocal of time, e.g. d^{-1} or hr^{-1}. The units of k_2 are reciprocal time, i.e. d^{-1} or hr^{-1}.

The organism-water kinetic model views bioconcentration as a balance between two kinetic processes, i.e. chemical uptake from the water and elimination to the water. The differential equation describing this process is

$$dC_F/dt = k_1.C_W - k_2.C_F \tag{3.2}$$

or

$$V_F.dC_F/dt = k_1.V_F.C_W - k_2.V_F.C_F \tag{3.3}$$

where V_F is the volume of the fish, which can be expressed in either units of volume (e.g. ml) or mass (e.g. grams). The term $V_F.dC_F/dt$ represents the net flux of chemical (e.g. in moles of chemical per unit time) into the organism. This net flux is the result of total flux of chemical from the water into the organisms, i.e. $k_1.V_F.C_W$ and the total flux of chemical from the organism to the water, i.e. $k_2.V_F.C_F$.

If the water concentration C_W is constant, this differential equation can be integrated to give the following equation for the concentration in the organism

$$C_F = C_W.(k_1/k_2).(1-\exp(-k_2.t)) \tag{3.4}$$

This equation describes the chemical concentration in the organism with time when an uncontaminated organism, with no initial chemical concentration, is exposed to water with a constant concentration of the chemical for a certain length of time. The time function of the chemical concentration in the organism is illustrated in Figure 3.1.

Figure 3.1 demonstrates that at the beginning of the exposure period C_F increases rapidly as a result of a large net uptake of chemical in the fish i.e. $k_1.C_W > k_2.C_F$. However, after a certain time the concentration in the organism does not further increase. In other words, the net uptake of chemical into the organism, i.e. dC_F/dt, becomes zero. This condition is often referred to as "steady-state". Theoretically steady-state is achieved at infinite exposure time, but in reality steady-state can often be reached within the duration of the experiment. From equation 3.4 it follows, that at steady-state the ratio of the chemical concentrations in the organism and water reaches a constant value, equal to the ratio of the uptake and elimination rate constants i.e.,

$$K_C = C_F/C_W = k_1/k_2 \tag{3.5}$$

If this ratio is larger than 1 bioconcentration will occur and k_1/k_2 is the bioconcentration factor K_C. The bioconcentration factor is thus viewed as the ratio of the rate constants for chemical uptake and elimination. It is usually treated as a dimensionless quantity, but in fact it has units of ml water per gram of organism. K_C is dimensionless only when the density of the organism is 1.0 gram per ml, which is often a reasonable estimate for aquatic organisms.

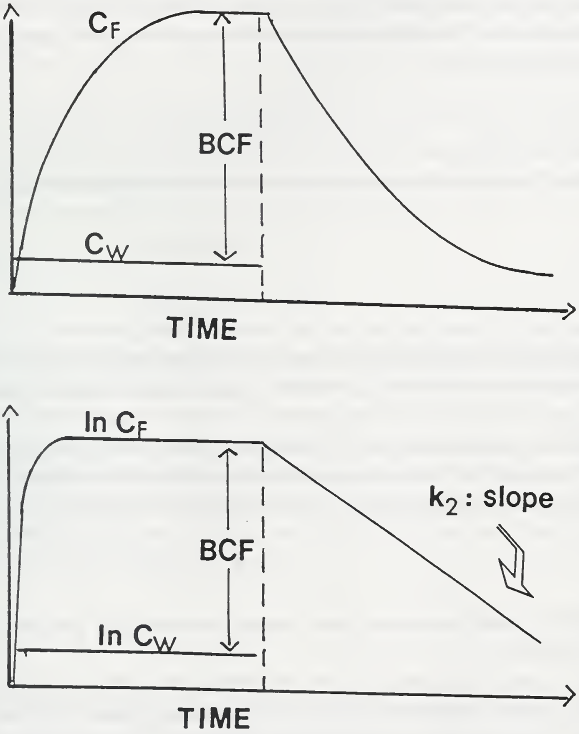


Figure 3.1

Illustrative example demonstrating that (i) -LEFT- when a fish is exposed to a chemical concentration in the water (C_W), the chemical concentration in the fish (C_F) increases exponentially with time until it reaches a constant concentration (i.e. steady-state has been achieved); (ii) -RIGHT- when a contaminated fish is exposed to clean, uncontaminated ($C_W = 0$) water the chemical concentration in the fish decreases exponentially with time.

Figure 3.1 further illustrates that when a contaminated organism is introduced into clean, uncontaminated water (C_W is zero) the organism will lose chemicals to the water resulting in a drop of C_F with time. The differential equation describing this process is again equation 3.2, but with a C_W of zero, i.e.

$$dC_F/dt = -k_2.C_F \tag{3.6}$$

which, after integration with an initial concentration in the organism of $C_{F,t=0}$ becomes

$$C_F = C_{F,t=0}.\{\exp(-k_2.t)\} \tag{3.7}$$

or

$$\ln C_F = \ln C_{F,t=0} - k_2.t \tag{3.8}$$

Equation 3.8 demonstrates that in a logarithmic plot, ln C_F decreases linearly with time. The slope of this plot is the elimination rate constant k_2 and has units of reciprocal time.

The rate at which chemicals are eliminated by organisms can also be expressed by the biological half-time $t_{1/2}$. This is the time required to reach half the initial concentration in the organism when exposed to uncontaminated water. It follows from equation 3.8 that the biological half-time, $t_{1/2}$ is

$$t_{1/2} = \ln 2/k_2 = 0.693/k_2 \tag{3.9}$$

This kinetic model incorporates several assumptions regarding the mechanism of chemical uptake and depuration i.e.

(i) Uptake and elimination involve chemical transfer processes, which are passive in nature. Chemical transfer is thus believed to be controlled by passive diffusion and/or fluid flows such as gill ventilation or blood flow. Active, enzyme-mediated transfer, for example across membranes, is considered unimportant.

(ii) There is no metabolic transformation of the chemical in the organism.

(iii) The organism is considered to be a single homogeneous compartment, i.e. all the chemical within the organism is equally "accessible".

As mentioned earlier, a large number of experiments involving different species of organisms and various unmetabolizable organic chemicals has provided support for this model. For example, as will be discussed in more detail in section 3.3, organic chemicals tend to distribute homogeneously throughout the lipids of the organism. Chemical concentrations in the lipid fraction of fish muscle tissue thus tends to be approximately equal to the chemical concentrations in the lipids of other tissues in the fish. This indicates that it is often acceptable to consider a fish a homogeneous compartment. Within the limits of experimental error, it can thus be concluded that the inherent assumptions of the two compartment kinetic model are generally justified. When more detailed experimental data become available this model can be accordingly improved.

Fugacity Models

Fugacity models, introduced and applied by Mackay and coworkers, are useful for describing and modelling the behaviour or fate of chemicals in the environment (Mackay and Paterson 1982). Ultimately, they are algebraically equivalent to the conventional concentration models, thus either can be used. The fugacity approach is based on the concept that when a substance is introduced into a system, consisting of phases of different chemical nature, it will tend to reach a thermodynamic equilibrium between all the phases, given enough time and assuming no chemical degradation occurs. At equilibrium the chemical's fugacity (f) in all phases are equal. For an organism-water system at equilibrium, this implies that the chemical's fugacities in the organism f_F and in the water f_W are equal i.e.

$$f_F = f_W \tag{3.10}$$

Fugacity reflects (but is not equal to) the chemical's concentration. It has units of pressure, usually expressed in the SI unit of pascals (Pa) and it is often viewed as the tendency of a chemical to leave a phase. Similar to the tendency of an object of high temperature to lose heat to an object of lower temperature, phases with high chemical fugacities tend to lose chemical to phases with a lower chemical fugacity. Given enough time, phases will reach equal fugacities similarly to objects reaching the same temperature.

Fugacity is related to concentration C through a fugacity capacity term Z i.e. C equals f.Z. The fugacity capacity, which has units of moles per m^3 per Pa (i.e. $moles.m^{-3}.Pa^{-1}$), reflects the capacity of the phase to accommodate the chemical. It is therefore a property of the chemical and the phase, reflecting the phase's capacity to "dissolve" the chemical. It can be compared with the heat capacity, which is the capacity of an object to store heat. The fugacity approach has been explained in detail by Mackay and Paterson (1982). We refer to these authors for a more detailed account of fugacity and its applications in environmental modelling.

Since f_F and f_W equal respectively C_F/Z_F and C_W/Z_W, equation 3.10 can be rewritten as

$$K_C = C_F/C_W = Z_F/Z_W \tag{3.11}$$

This equation demonstrates that at equilibrium the ratio of concentrations in fish and water reflects the ratio of the chemical's fugacity capacities. It thus establishes the link between chemical bioconcentration in fish and the chemical's thermodynamically controlled tendency to partition between water and the organism. This bioconcentration model views bioconcentration as a chemical partitioning process and the bioconcentration process as a partition coefficient, which is controlled by the relative affinities of the chemical for the water and the tissue of the organism. This concept is of great importance since it provides the basis for correlations between the bioconcentration factor (i.e. an organism-water partition coefficient) and the 1-octanol-water partition coefficient. This will be discussed in more detail in section 3.3.

Following the fugacity approach chemical exchange between the organism and the water can be described by

$$V_F.Z_F.df_F/dt = D_F.(f_F - f_W) \tag{3.12}$$

where V_F is the volume of the organism, Z_F is the chemical's fugacity capacity in the organism, D_F is the transport parameter of chemical exchange between the organism and the water and t is time. The transport parameter D can reflect passive diffusion as expressed by the diffusivity or mass transfer coefficients or fluid flow (e.g. in gill ventilation or blood flow) as G.Z where G (m^3/s) is the volumetric fluid flow rate. Equation 3.12 is equivalent to equation 3.3. The chemical flux from the water to the organism is now $D_F.f_W$ and the total flux back to the water is $D_F.f_F$. The net flux is $V_F.Z_F.df_F/dt$. Integration of equation 3.12 with a constant f_W and an initial f_F of zero gives

$$f_F = f_W.\{1 - \exp(-D_F.t/V_F.Z_F)\} \tag{3.13}$$

Since f_F is C_F/Z_F and f_W is C_W/Z_W, equation 3.13 can be rewritten as

$$C_F = C_W.(Z_F/Z_W).\{1 - \exp(-D_F.t/V_F.Z_F)\} \tag{3.14}$$

which is equivalent to equation 3.4. Similar to equation 3.4, this equation establishes the variation of the chemical concentration in the organism with time. It shows that at steady-state, which is achieved at infinite exposure time t, the ratio of the concentrations in the organism and water or the bioconcentration factor K_C equals the ratio of the fugacity capacities in the organism and water i.e.

$$K_C = C_F/C_W = Z_F/Z_W \tag{3.15}$$

Equation 3.15, which is equal to equation 3.11, demonstrates that, although organisms absorb and eliminate chemicals from and to the water, the bioconcentration factor can be viewed as an organism-water partition coefficient, reflecting the chemical's affinity for the organism relative to that for water.

The fugacity and the kinetic description of the bioconcentration process are similar in that they are based on the same assumptions. These assumptions, which were discussed earlier, are that (i) chemical transfer is passive, (ii) there is no metabolic transformation and (iii) the organism is viewed as one, homogeneous compartment. The difference in the two approaches is that in the kinetic approach, bioconcentration is viewed as the balance between the rates of uptake and elimination, whereas in the fugacity approach bioconcentration is a chemical partitioning process between the organism and water.

The strength of the kinetic descriptions is that the rate constants can be measured directly from uptake and depuration experiments. The fugacity-equations, however, give an in-depth view of the actual mechanism of the bioconcentration process since they have the ability to distinguish between transport processes (characterized by transport parameters i.e. D) and partitioning phenomena (characterized by the fugacity capacities i.e. Z). The two approaches complement each other, and are best combined. This can be easily achieved by comparing equations 3.4 and 3.14 from which it follows that

$$k_1 = D_F/V_F.Z_W \tag{3.16}$$

$$k_2 = D_F/V_F.Z_F \tag{3.17}$$

$$K_C = k_1/k_2 = Z_F/Z_W \tag{3.18}$$

Equation 3.15 shows that bioconcentration is the result of a chemical partitioning process of the chemical between the organism and water, controlled by the relative affinities of the chemical for the organism and water. For a chemical to bioconcentrate in the absence of active transport, it must have a high affinity for non-aqueous phases in the organism. For organic chemicals this is usually the lipid phase of the organism. Generally, it is believed that only an insignificant fraction of the total chemical in the organism resides in the aqueous phases of the organism.

The fugacity capacity in the organism Z_F therefore represents (i) the relative amount of lipids in the organism, which is often expressed by the lipid fraction L_F, i.e. the ratio of the lipid volume V_L and V_F, V_L/V_F, and (ii) the fugacity capacity in the lipids Z_L. Z_F thus equals and can be replaced by $L_F.Z_L$. Equations 3.16 to 3.18 can therefore also be written as

$$k_1 = L_F.D_F/V_L.Z_W \tag{3.19}$$

$$k_2 = D_F/V_L.Z_L \tag{3.20}$$

$$K_C = k_1/k_2 = L_F.Z_L/Z_W = L_F.K_L \tag{3.21}$$

Equations 3.18 and 3.21 demonstrate that bioconcentration of organic chemicals can be viewed as a partitioning process of the chemical between the lipids of the organism and water. The bioconcentration factor K_C of a chemical in an organisms is therefore dependent on the lipid-water partition coefficient of the chemical i.e. K_L and on the lipid-content L_F of the organism. The bioconcentration factor K_C of a chemical is therefore higher in an organisms with a higher lipid content. However, the bioconcentration factor of a specific chemical expressed on a lipid weight basis i.e. K_L or K_C/L_F or Z_L/Z_W should be approximately similar in all organisms.

3.3 Relationship Between Lipid Content and Bioconcentration Factor

Since bioconcentration of organic chemicals can be viewed as a partitioning process of the chemical between the lipids of the organism and the water, it follows that the lipid content of the organism affects the contaminant concentration in the organism. Organisms with a high fraction of lipids have a larger capacity to store organic chemicals. Bioconcentration factors in organisms with a high lipid content are therefore usually higher than in organisms with a lower lipid content. This effect is described by the simple linear relationship

$$K_C = L_F.C_L/C_W = L_F.K_L \tag{3.22}$$

where L_F is the lipid content of the organism and C_L/C_W is the lipid-weight based bioconcentration factor (K_L).

The effect of the lipid content of organisms on the bioconcentration factor has been observed by many authors in laboratory experiments and in the field. Geyer et al. (1985) showed a linear increase of the bioconcentration factor (K_C) with lipid content for 1,2,4-trichlorobenzene (i.e. $K_C = 16{,}600\ L_F$ (n=26, r=0.873)) and lindane (i.e. K_C equals 16,050 L_F, n=68, r=0.99) in various fresh water fish species in laboratory experiments. When wet weight based bioconcentration factors were normalized for lipid content and expressed on a lipid-weight basis as K_L the variation between the bioconcentration factors in the fish species was significantly reduced.

Goerke (1984) showed that concentrations of 2,4,6,2',4',6'-hexachlorobiphenyl in different parts of the sandworm (Nereis virens) increased with increasing lipid content. Nimmo et al. (1970, 1971) reported that the internal distribution of DDT and PCBs in the various tissues of the pink shrimp (Penaeus duorarum) corresponded with the lipid content of the tissues. Ernst et al. (1976) reached similar conclusions for DDT and PCBs in three fish species in the English Channel, including the yellow gurnard (Trigla lucerna). It was shown that when tissue concentrations were expressed on a lipid weight basis, contaminant concentrations were approximately similar. Holden (1962) found that the distribution of ^{14}C-DDT among the tissues of brown trout was directly related to the lipid content of the tissues. Studies with PCBs in carp Cyprinus carpio (Yoshida et al. 1973) and lindane in rainbow trout, Salmo gairdneri (Tooby and Durbin, 1975) showed similar results. Holden and Marsden (1967) showed that DDT concentrations in various tissues of a single porpoise (Phocaena) differed considerably e.g. concentrations in the blubber were almost 100 times higher than in the kidney when expressed on a wet weight basis but only 2 times when expressed on a lipid weight basis. The brain was the only exception having a lipid based contaminant concentration which was 10 times less than the other tissues. Reinert and Bergman (1974) also showed variations in the DDT concentrations between various tissues of coho salmon (Oncorhynchus kisutch). However, when DDT concentrations were expressed on a lipid weight basis differences between the tissue concentrations were insignificant.

It can thus be concluded that organic chemicals have a tendency to partition exclusively in lipids. They further distribute homogeneously among the lipids of the various tissues in fish. The main factors which are known to affect the amount of total lipids in aquatic biota are season and diet. The variation in lipid content with season is related to the sexual cycle and the water temperature. In general, before and during gonad development the lipid content in aquatic biota increases and during spawning it decreases. This usually results in an up-and-down cycle throughout the year. Diet is also an important factor controlling lipid amounts in aquatic biota. Starvation results in a progressive depletion of the lipid pool, whereas an abundance of food may lead to higher lipid levels.

The effect of varying lipid levels on contaminant concentrations and bioconcentration factors in biota is complex. Equation 3.22 demonstrates that organisms of a lower lipid content will also have a lower bioconcentration factor K_C and thus a lower wet weight based chemical concentration. In general, this is true but it may not always apply to organisms which have been losing lipids due to a change in season or other reasons. A reduction of the lipid content of the organism will result in a net loss of the chemical and a drop in concentration in the organism while the chemical is achieving a new chemical equilibrium with the water. However, this drop in concentration can only be achieved when the chemical is actually eliminated. When chemical elimination is slow and slower than the loss rate of lipids, the reduction in the lipid volume of the organism results in an increase of the chemical concentration in the lipids (C_L). This appears to be the case for very hydrophobic chemicals such as DDT and PCB. For example, Reinert and Bergman (1974) measured DDT concentrations and lipid contents in coho salmon (*Oncorhynchus kisutch*) of Lake Michigan and showed that from August to the end of January the lipid content in the fish decreased from 13.2 % to 2.8 % causing an increase in DDT concentration on a lipid weight basis from 93.2 to 439.3 mg/kg. Since the elimination of DDT from the fish is extremely slow, the total amount of DDT in the organism remained approximately constant. But since the same amount of chemical is now present in a smaller amount of lipid the chemical concentration in the lipids is higher. Conversely, it can be expected that an increase in the lipid content will result in a reduction of the chemical concentration in the lipid when uptake of the chemical is slower than the increase in lipid content.

3.4 Relationship Between the Bioconcentration Factor and the 1-Octanol-Water Partition Coefficient

It has been shown that in the absence of metabolic transformation, bioconcentration is the result of a thermodynamically controlled partitioning process of the chemical between the lipids of the organism and water. This partitioning process can be described by a wet weight based bioconcentration factor K_C or a lipid weight based bioconcentration factor K_L. Since the lipids are only a fraction of the organism, the bioconcentration factor K_C, i.e. C_F/C_W, is only a fraction of K_L or C_L/C_W. This fraction is the lipid fraction or lipid content of the organism L_F, i.e. K_C equals $L_F.K_L$.

In general, a partition coefficient expresses the relative affinities of a chemical for the two phases involved. In the case of bioconcentration these phases are the water and the lipid tissue of the organism. The affinity of a chemical for the two phases can be expressed by the chemical's activity coefficient, solubility or fugacity capacity. The lipid weight based bioconcentration factor, which is actually a lipid-water partition coefficient can therefore be viewed as the ratio of the chemical's solubilities in lipids (S_L) and water (S_W) or as the ratio of the fugacity capacities in the lipids (Z_L) and water (Z_W) i.e.

$$K_L = C_L/C_W \; Z_L/Z_W = S_L/S_W \tag{3.22A}$$

Since the lipid content varies considerably between organisms, the bioconcentration factor (i.e. K_C) of a certain compound also tends to vary (Geyer et al. 1985). The lipid weight based bioconcentration factor (i.e. K_L), however, tends to be constant. This is due to the fact that, although organisms have a somewhat different lipid composition, the solubility of organic chemicals in various lipids is approximately similar. The lipid weight based bioconcentration factor can thus be viewed as a property of the chemical itself, relating to its ability to partition between lipids and water.

In pharmaceutical chemistry the tendency of chemicals to partition between lipids and water is traditionally expressed by the chemical's partition coefficient between 1-octanol and water i.e.

$$K_{OW} = C_O/C_W = Z_O/Z_W = S_O/S_W \tag{3.23}$$

where C_O and C_W are the concentrations of the chemical in 1-octanol and water respectively, at equilibrium. This concept was introduced by Hansch and Leo (1979), who selected 1-octanol as a surrogate phase for lipids because the ratio of carbon, oxygen and hydrogen atoms in 1-octanol matched that of the common lipid glyceryltrioleate. It was thus believed that the solubility of chemicals in 1-octanol and lipids are approximately similar and that 1-octanol-water and lipid-water partition coefficients are also similar.

The 1-octanol-water partition coefficient has been shown to be a very successful predictor of a variety of drug activities, such as narcosis, toxicities and distribution processes, all of which are controlled by the drug's partitioning tendency in "lipid" target sites in organisms (Leo and Hansch 1972, Dearden 1985, Seeman 1972, Veith et al. 1983). Neely (1979) and Veith et al. (1979) demonstrated that bioconcentration factors in fish can also be successfully related to the 1-octanol-water partition coefficient. These authors showed excellent linear correlations between log K_c and log K_{ow} in fish. Numerous authors have subsequently reported similar linear correlations in various aquatic organisms. These linear correlations demonstrate that chemicals with high 1-octanol-water partition coefficients have high bioconcentration factors and that chemicals with low 1-octanol-water partition coefficients have a relatively low tendency to bioconcentrate in organisms.

The observed linear correlations between log K_c and log K_{ow} have important implications. First of all, they provide strong support for the hypothesis that bioconcentration is a simple lipid-water partition process. Secondly, they allow an aquatic organism to be viewed as a droplet of 1-octanol in water and bioconcentration to be treated as a 1-octanol-water partition process. This provides a most practical tool for assessing the bioconcentration tendency of organic chemicals, without having to perform the fairly elaborate bioconcentration experiments. After the relationship between the bioconcentration factor and the 1-octanol-water partition coefficient has been established, a simple measurement of the 1-octanol-water partition coefficient can give information about the chemical's potential to bioconcentrate in various aquatic organisms.

Knowledge and understanding of relationships between bioconcentration and chemical properties (such as the 1-octanol-water partition coefficient) and the factors affecting these correlations is therefore crucial for any exposure and hazard assessment of organic contaminants in aquatic ecosystems.

Observed Relationships Between log K_c and log K_{ow}

In order to establish a link between the bioconcentration factor and chemical properties, several authors have investigated the relationship between the bioconcentration factor and the 1-octanol-water partition coefficient. These relationships are primarily studied under laboratory conditions, since organisms in the field are not solely exposed to organic contaminants from the water but also from other sources (e.g. food). Since 1-octanol-water partition coefficients and bioconcentration factors often vary by several orders of magnitude, resulting in a non-normal distribution of the data, K_c-K_{ow} relationships are usually expressed on a logarithmic basis. Most common is the linear correlation between the logarithms of the bioconcentration factor (log K_c) and the 1-octanol-water partition coefficient (log K_{ow}) i.e.

$$\log K_c = A.\log K_{ow} + B \tag{3.24}$$

where A and B are empirical constants, which can be derived by linear regression of log K_c and log K_{ow} data. Since equation 3.24 can be rewritten as

$$K_c = B.K_{ow}{}^{A} \tag{3.25}$$

it follows that only when A equals 1.0 the bioconcentration factor and the 1-octanol-water partition coefficient are actually linearly related. If A is not 1.0, the 1-octanol-water partition coefficient and the bioconcentration factor follow a non linear relationship.

Table 3.1 gives an overview of reported linear correlations between log K_c and log K_{ow} for a variety of organic chemicals in various aquatic organisms and demonstrates that the observed correlations between log K_c and log K_{ow} in aquatic organisms vary considerably in slope and intercept. The reported slopes vary from as low as 0.28 to as high as 1.53 and are rarely equal to 1.0.

The reported intercepts also vary considerably i.e. from -3.03 to 2.60. Differences in the lipid content between the organisms may contribute to these variations. For a comparison of bioconcentration in various organisms, it is therefore preferable to normalize the bioconcentration factors with respect to lipid content by expressing the bioconcentration factor on a lipid weight basis as K_L. This enables a direct comparison of bioconcentration factors between different organisms. Unfortunately, however, the lipid content of the test organism is often not reported.

The large variation between observed log K_c-log K_{ow} relationships makes it difficult to select a correlation which could be used for predictive purposes. It also indicates that a lipid-water partitioning process may somewhat oversimplify the bioconcentration phenomenon. In section 3.5 we will therefore discuss some of the factors which affect the bioconcentration process.

In order to develop a general correlation between the 1-octanol-water partition coefficient and the bioconcentration factor, virtually all reported bioconcentration factors were compiled and plotted versus the 1-octanol-water partition coefficient in Figure 3.2. Linear regression of the log K_{ow} versus log K_c data resulted in the following correlation

$$\log K_c = 0.57\ (+/-\ 0.36).\log K_{ow} + 0.42\ (+/-\ 1.17) \tag{3.26}$$

$$n = 426,\ r^2 = 0.57$$

Figure 3.2 and equation 3.26 demonstrate the linearity between log K_c and log K_{ow}. The large number of data points considered, compensates for outliers, which would have a dominant influence on correlations performed on smaller data sets. Since equation 3.26 is a correlation for the bioconcentration factor on a wet weight basis it represents an average in the lipid content of the various test organisms.

Relationships between log K_c and log K_{ow} are often reported to be non-linear for chemicals of very high K_{ow} (log K_{ow} > 6). Sugiura et al. (1978) observed that the bioconcentration factors of polychlorinated and polybrominated biphenyls in the killifish (Oryzias latipes) were proportional to the 1-octanol-water partition coefficient for chemicals with a log K_{ow} below 6 but not for chemicals with higher K_{ow}. Bruggeman et al. (1984) reported that bioconcentration factors of polychlorinated biphenyls in the guppy (Poecilia reticulata) increased with increasing log K_{ow} up to a log K_{ow} of 6 but then declined for chemicals of higher K_{ow}. A maximum bioconcentration factor was observed for tetra- and hexachlorobiphenyls, which have a log K_{ow} of approximately 6 to 7. Opperhuizen et al. (1985) showed a similar loss of linear correlation for polychlorinated naphthalenes in the guppy.

Gobas et al. (1989) showed a parabolic relationship between the logarithms of the observed lipid weight based bioconcentration factors and the 1-octanol-water partition coefficient in the guppy

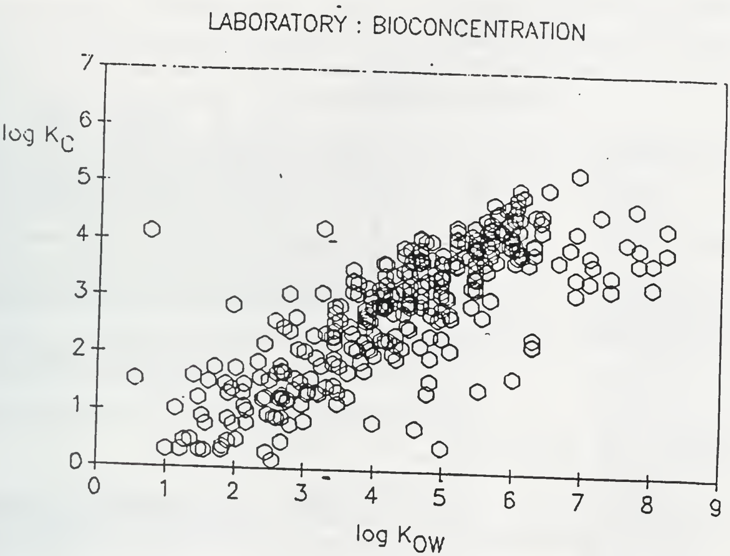


Figure 3.2

The logarithm of the wet weight based bioconcentration factor (log K_c) of 426 organic chemicals, including halogenated hydrocarbons, polynuclear aromatics and halogenated alkanes in various aquatic organisms versus the logarithm of the 1-octanol-water partition coefficient (log K_{ow}).

Table 3.1

Reported linear correlations between the logarithm of the wet weight based bioconcentration factor ($\log K_c$) or lipid weight based bioconcentration factor ($\log K_L$) and the logarithm of the 1-octanol-water partition coefficient in various fish species; n is the number of chemicals considered in the correlation, r is the correlation coefficient.

Organism	Correlation	n	r^2	Reference
Algae (Chlorela fusca)	$\log K_c = 0.68 \cdot \log K_{ow} + 0.16$	41	0.81	Geyer et al.,1984
Algae (Scenedesmus acutus)	$\log K_c = 0.70 \cdot \log K_{ow} - 0.26$	8	0.93	Ellgehausen et al. 1982
Algae (Selenastrum capri.)	$\log K_c = 0.28 \cdot \log K_{ow} + 2.60$	9	0.64	Mailhot 1987
Catfish (Ictalurus melas)	$\log K_c = 0.83 \cdot \log K_{ow} - 1.71$	7	0.97	Ellgehausen et al. 1982
Daphnia magna	$\log K_c = 0.71 \cdot \log K_{ow} - 1.26$	8	0.94	Ellgehausen et al. 1982
Fathead minnow	$\log K_c = 0.86 \cdot \log K_{ow} - 0.72$	54	0.86	Veith et al.,1980
Guppy	$\log K_l = 0.91 \cdot \log K_{ow} + 0.65$	20	0.61	Gobas et al. 1989
Guppy	$\log K_c = 0.98 \cdot \log K_{ow} - 0.06$	5	0.93	Konemann & Van Leeuwen 1980
Guppy	$\log K_c = 1.02 \cdot \log K_{ow} - 1.82$			Saarikoski & Viluksela 1982 cited in Butte et al. 1987
Killifish	$\log K_c = 0.64 \cdot \log K_{ow} - 0.77$	4	0.92	Saski et al.,1982
Mussel (Mytilus edulis)	$\log K_c = 0.86 \cdot \log K_{ow} - 0.81$	16	0.91	Geyer et al.,1982
Pinfish	$\log K_c = 0.65 \cdot \log K_{ow} + 0.14$	10	0.77	Zaroogian et al.,1985
Rainbow trout	$\log K_c = 0.54 \cdot \log K_{ow} + 0.12$	8	0.90	Neely et al.,1974
Rainbow trout	$\log K_c = 0.94 \cdot \log K_{ow} - 0.68$	18	0.91	Oliver 1984
Rainbow trout	$\log K_c = 1.02 \cdot \log K_{ow} - 0.63$	11	0.99	Oliver & Niimi 1983
Rainbow trout	$\log K_c = 1.00 \cdot \log K_{ow} - 0.87$	11	0.99	Oliver & Niimi 1983
Rainbow trout	$\log K_c = 0.99 \cdot \log K_{ow} - 1.02$	7	0.79	Oliver & Niimi 1984
Rainbow trout	$\log K_c = 0.96 \cdot \log K_{ow} - 0.56$	16	0.95	Oliver & Niimi 1985
Sheepshead minnow	$\log K_c = 0.50 \cdot \log K_{ow} + 0.78$	14	0.42	Zaroogian et al.,1985
Topmouth gudgeon	$\log K_c = 1.53 \cdot \log K_{ow} - 3.03$	15	0.71	Kanazawa 1981
Various	$\log K_c = 0.60 \cdot \log K_{ow} + 0.19$	33	0.56	Davies & Dobbs 1984
Various	$\log K_c = 0.80 \cdot \log K_{ow} - 0.52$	107	0.82	Isnard & Lambert 1988
Various	$\log K_c = 0.94 \cdot \log K_{ow} - 1.50$	26	0.76	Kenaga & Goring 1980
Various	$\log K_c = 1.00 \cdot \log K_{ow} - 1.32$	44	0.95	Mackay 1982
Various	$\log K_c = 0.85 \cdot \log K_{ow} - 0.70$	59	0.90	Veith et al.,1979
Various	$\log K_c = 0.76 \cdot \log K_{ow} - 0.23$	84	0.82	Veith et al.,1980
Various	$\log K_c = 0.79 \cdot \log K_{ow} - 0.40$	90	0.86	Veith & Kosian 1983
Zebra fish	$\log K_c = 0.58 \cdot \log K_{ow} + 0.12$	10	0.89	Butte et al. 1987

(*Poecilia reticulata*). For chemicals of log K_{ow} below 6, log K_L followed a linear relationship with log K_{ow}. But bioconcentration factors of higher log K_{ow} chemicals (i.e. larger than 6) were considerably lower than expected from the linear correlation with log K_{ow} and even showed a tendency to fall with increasing K_{ow}. Figure 3.3 illustrates these observations.

Muir et al. (1985) showed a similar behaviour of the bioconcentration factor in the rainbow trout (*Salmo gairdneri*) and proposed a parabolic relationship between log K_C and log K_{ow} i.e.

$$\log K_C = 2.44\ (+/-\ 0.24).\log K_{ow} - 0.19\ (+/-\ 0.02).(\log K_{ow})^2 + 3.47 \qquad (3.27)$$

Because of a lack of theoretical basis for parabolic relationships, Muir et al. (1985) also proposed a bilinear correlation to represent the behaviour of bioconcentration factor with the 1-octanol-water partition coefficient i.e.

$$\begin{gathered}\log K_C = 0.95\ (+/-\ 0.08).\log K_{ow}\ - \\ 0.62\ (+/-\ 0.05).\{\log K_{ow} - \log K_{ow,max}\} - 0.78\ (+/-\ 0.41)\end{gathered} \qquad (3.28)$$

where $K_{ow,max}$ is the 1-octanol-water partition coefficient for which a maximum bioconcentration factor was observed and the term {log K_{ow} - log $K_{ow,max}$} equals zero when log K_{ow} is smaller than $K_{ow,max}$.

3.5 Factors Affecting Relationships Between the Bioconcentration Factor and the 1-Octanol-Water Partition Coefficient

Table 3.1 demonstrates that observed relationships between the bioconcentration factor and the 1-octanol-water partition coefficient vary considerably. Even when bioconcentration factors are expressed on a lipid weight basis as K_L, there is a relatively large scatter in the data resulting in large error bars in the slope and intercept of the log K_L-log K_{ow} relationship (Gobas et al. 1989). This suggests that the concept of lipid-water partitioning may be an oversimplification of the actual bioconcentration process or that 1-octanol-water partitioning is not a satisfactory analogue of lipid-water partitioning in aquatic biota. It also makes estimation of the bioconcentration factor, based on the 1-octanol-water partition coefficient, a difficult task.

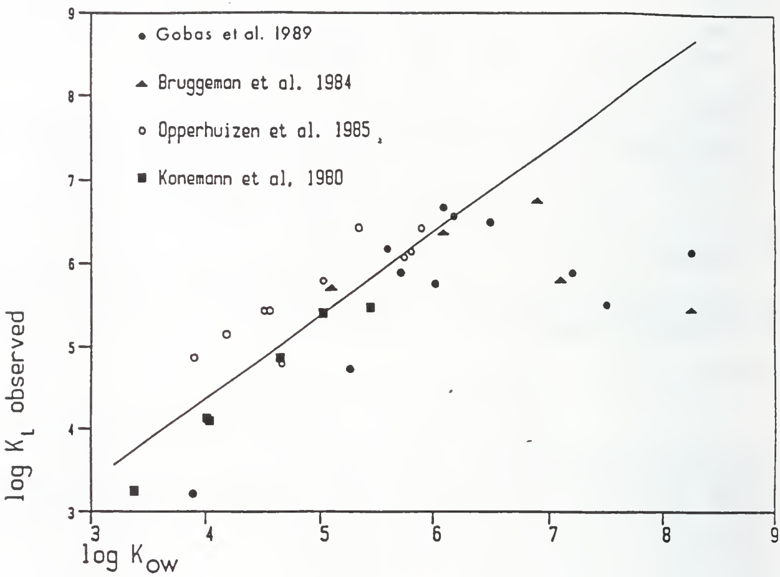


Figure 3.3

The logarithm of observed lipid weight based bioconcentration factors (log K_L) of chlorinated and brominated benzenes and biphenyls in the guppy (Poecilia reticulata) versus the logarithm of the 1-octanol-water partition coefficient (log K_{OW}). The solid line represents the linear correlation for chemicals with a log K_{OW} below 6.5.

Various factors and experimental errors have been shown, or suggested, to affect relationships between the bioconcentration factor and the 1-octanol-water partition coefficient. These factors are (i) metabolic transformation of the chemical in the organism, (ii) membrane permeation rates, (iii) duration of the experimental exposure time, (iv) bioavailability, (v) chemical elimination in faecal matter and (vi) a loss of similarity between lipid-water and 1-octanol-water partitioning. We review and discuss each of these factors.

Metabolic Transformation

Metabolic transformation is the process by which chemicals are enzymatically converted by organisms resulting in the formation of new (transformation) product(s) or metabolite(s), with a loss of the parent compound. The metabolite has physical and chemical properties different from that of the parent compound. The fate of the metabolite, within the organism, with respect to internal distribution, bioaccumulation and routes and rates of excretion and transformation differs from that of the parent compound. Metabolites are usually either more or less toxic than the parent compound, i.e. metabolic transformation is either an activation or detoxification process. For example, Anjum and Qadri (1986) showed that in Tilapia mossambica fenitrothion is transformed into fenitrooxon, which is more toxic than fenitrothion. On the other hand, Lech (1974) demonstrated a detoxification of 3-trifluoromethyl-4-nitrophenol in the rainbow trout, as a result of metabolic transformation.

Metabolic transformation usually results in metabolic products that can be more quickly eliminated by the organism than the parent compound. However, in some cases the metabolites have been shown to accumulate to a greater extent than the parent compound. For example, Spacie et al. (1983) showed that the metabolites of naphthalene and benzo-a-pyrene have higher bioconcentration factors than the parent compounds.

Metabolic transformation can be viewed as an elimination route for (parent) chemicals absorbed by organisms. It enhances the rate of loss of the parent compound from the organism and thus changes the balance between the rates of chemical uptake and depuration in favour of chemical depuration, resulting in lower bioconcentration factors. For example, in the gold fish pentachlorophenol is metabolized to a glucuronide conjugate, which is quickly excreted (Glickman et al. 1977). Metabolic transformation thus prevents the build-up of high concentrations of pentachlorophenol in the gold fish and lowers the bioconcentration factor.

The effect of metabolic transformation on bioconcentration and the bioconcentration factors in organisms can be quantified by extending the organism-water two compartment model to include a term for metabolic transformation. If the rate of metabolic transformation can be represented by a first order metabolic rate constant k_R, the following differential equation gives a description of the bioconcentration process in presence of metabolic transformation:

$$dC_F/dt = k_1.C_W - k_2.C_F - k_R.C_F \tag{3.29}$$

Integration of this differential equation, assuming a constant chemical concentration in the water, results in

$$C_F = C_W.(k_1/(k_2 + k_R)).\{1 - \exp(-[k_2 + k_R].t)\} \tag{3.30}$$

Equation 3.30 demonstrates that at steady state (i.e. infinite exposure time) the bioconcentration factor can be expressed as

$$K_C = C_F/C_W = k_1/(k_2 + k_R) \tag{3.31}$$

Comparison of equation 3.21, for bioconcentration without metabolic transformation, and equation 3.30 shows that metabolic transformation reduces the bioconcentration factor.

The effect of metabolic transformation on organism-water partitioning and the relationship between the bioconcentration factor and the 1-octanol-water partition coefficient is best demonstrated by writing equations 3.29 to 3.31 in fugacity expressions. Equation 3.29 is equivalent to

$$V_F.Z_F.dC_F/dt = D_F.f_W - D_F.f_F - D_R.f_F \tag{3.32}$$

where D_R is the transformation parameter for metabolic conversion, i.e. $V_FZ_Fk_R$. Upon integration equation 3.32 becomes

$$C_F = C_W.(Z_F/Z_W).(D_F/(D_F + D_R).\{1 - \exp(-[D_F + D_R].t)\} \tag{3.33}$$

which shows that at steady state the bioconcentration factor can be defined as

$$K_C = C_F/C_W = (Z_F/Z_W).(D_F/(D_F + D_R) \tag{3.34}$$

Equation 3.34 shows that if a chemical is being transformed, the bioconcentration factor is not solely controlled by the thermodynamic quantities Z_F and Z_W, which represent organism-water partitioning by Z_F/Z_W, but also by the relative rates of chemical elimination to the water and metabolic transformation. Metabolic transformation will therefore reduce the bioconcentration factor below the organism-water partition coefficient Z_F/Z_W, which is represented by the 1-octanol-water partition coefficient Z_O/Z_W.

Metabolic Transformation in Aquatic Organisms

Metabolic reactions of xenobiotics in aquatic organisms are generally classified in two groups i.e. Phase I and Phase II reactions. Phase I transformations involve oxidation, reduction or hydrolysis of the parent compound. Phase II transformations involve the addition or conjugation of specific groups to the parent compound or to the metabolic product(s) of the parent compound resulting from a phase I transformation.

Phase I Transformations

Oxidation of xenobiotics in aquatic organisms involves a series of reactions such as (i) epoxidation, (ii) aromatic hydroxylation, (iii) O-dealkylation, (iv) N-dealkylation, aliphatic hydroxylation, (v) decarboxylation, (vi) sulphuroxidation, (vii) desulphonation and (viii) dehydrohalogenation.

A group of hemoproteins, generally referred to as cytochrome P-450, are primarily responsible for these oxidative reactions. Cytochrome P-450 is located in the smooth endoplasmatic reticulum of cells. The highest activities of this enzyme system is usually found in the liver and the kidneys of various organisms. Most oxidative metabolic transformations therefore take place in the liver and the kidney.

The amounts of cytochrome P-450 in various organisms varies considerably. Sijm and Opperhuizen (1988) recently reviewed cytochrome P-450 levels in various organisms. Table 3.2, which summarizes their findings, shows that cytochrome P-450 levels vary from 0.009 (in the Killifish) to 1.03 nmoles Cyt. P-450/mg microsomal liver protein (in the Kissing Gourami). Average cytochrome

Table 3.2

Cytochrome-P450 levels in nmoles Cyt. P450/mg microsomal liver protein in various aquatic organisms.

SPECIES	CYTOCHROME-P450 (nmoles/mg mic protein)
Archosargus probatocephalus	0.18 - 0.44
Astacus astacus L.	0.31
Brachydanio rerio	0.30
Citharichtys stigneaus	0.08
Coregonus albula	0.05 - 0.07
Cyprinus carpio	0.27 - 0.38
Dasyatis sabina	0.43 - 0.50
Dasyatis sayi	0.30 - 0.32
Esox lucius	0.14
Fundulus parvipinnis	0.35
Ginglymostoma cirratum	0.47
Helostoma temmincki	1.03
Ictalurus melas	0.16
Ictalurus punctatus	0.15 - 0.24
Lepomis macrochirus	0.28 - 0.81
Leuciscus idus	0.18
Lutjanus griseus	0.25
Mugil cephalus	0.28 - 0.47
Oryzias latipes	0.01 - 0.37
Oryzias latipes	0.80
Paralichtys lethostigma	0.11
Perca fluviatilis L.	0.05
Poecilia reticulata	0.58
Pogonias cromis	0.14 - 0.15
Pseudopleuronectes americanus	0.12 - 0.85
Raja erinacea	0.22 - 0.32
Raja occellata	0.39 - 0.41
Rutilis rutilis L.	0.09
Salmo gairdneri	0.11 - 0.42
Salmo salar	0.84
Salmo trutta	0.20 - 0.40
Salmo trutta lacustris	0.17
Salvelinus fontinalis	0.15 - 0.56
Squalus acanthias	0.22 - 0.29
Stenotomus chrysops	0.35 - 0.62

P-450 levels in aquatic organisms are approximately 0.30 nmoles Cyt. P-450/mg microsomal protein, which are approximately 30 to 60 % of those in rats and 20 % of those in rabbits (Sijm and Opperhuizen 1988). Crustaceans and fish have approximately equal cytochrome P-450 levels. The rainbow trout, which is often used in bioaccumulation and toxicity experiments, has cytochrome P-450 levels that are somewhat lower than those in other fish species. The guppy, which has often been used in bioaccumulation studies, has cytochrome P-450 levels which are somewhat higher than the "average" level.

Although a direct relationship, between cytochrome P-450 levels in organisms and oxidation rates, has not been established, it is expected that organisms with higher cytochrome P-450 levels also have a higher oxidative capacity. And, a larger oxidative capacity may result in lower bioconcentration factors of the parent compound. It is thus possible that, for metabolizable chemicals, the choice of fish can influence the results of bioconcentration experiments and toxicity studies.

Hydrolysis

Little is known about hydrolytic reactions in aquatic organisms. Hydrolytic enzymes are esterases, amidases and epoxidehydrases. They occur in several organs (Lech and Vodicnic 1985, Bend and James 1978, Brodie et al. 1958) and are involved in the hydrolysis of amides, phosphates, carbamate esters, alkene-oxides and arene-oxides.

Reduction

As with hydrolytic reactions, there is very limited information on reductive processes in aquatic organisms. Enzymes, which are known to catalyze reduction reactions, are dechlorinative reductase, ketone reductase, alkene-oxide reductase, arene-oxide reductase, N-oxide reductase, nitro-reductase and azo-reductase. Dechlorination has been observed in marine species, but rates are usually very low (Bend and James 1978). Generally, reductases are active under anaerobic conditions and have low activities under aerobic conditions.

Phase II Reactions

As discussed in detail by Lech and Vodicnic (1985) and Bend and James (1978), conjugation (i.e. Phase II reactions) can occur if the parent compound, or its phase I metabolite, has either a -COOH, -OH or $-NH_2$ group. The main conjugation reactions are (i) glucuronic acid conjugation, (ii) gluthathion conjugation, (iii) sulphate conjugation, (iv) acetyl conjugation and (v) methyl conjugation.

Glucuronic acid conjugation involves a two step mechanism. First, uridinephosphoglucuronic acid (UDPGA) is synthesized from uridinediphospho-b-D-glucose. Secondly, glucuronic acid is transferred from UDPGA to the substrate. The glucuronic acid transfer to the substrate is catalyzed by glucuronyltransferase (UDPGA-t), a microsomal protein. UDPGA-t activities are often measured and reported in terms of the conjugation rate of p-nitrophenol (PNP). UDPGA-t activities in fish have been reported to range from 0.18 to 0.80 nmoles PNP conjugated per mg cytosolic protein per minute.

UDPGA-t activities have been reported to be temperature sensitive and inducible by various inducers.

Studies addressing the effect of glucuronic acid conjugation on bioaccumulation are rare. Muir et al. (1986) reported that glucuronic acid conjugation is an important depuration route for some chlorinated dibenzo-p-dioxins. This would explain the relatively low bioconcentration factors of the chlorinated dibenzo-p-dioxins.

Membrane Permeation

To explain the observed relatively low bioconcentration factors of some high K_{ow} chemicals and the resulting loss of linear correlation between the bioconcentration factor and the 1-octanol-water partition coefficient for high K_{ow} chemicals, various authors have suggested that molecular size may interfere with membrane permeation, gill uptake and bioconcentration (Moser and Anliker 1987, Muir et al. 1985, Kenaga and Goring 1980, Tulp and Hutzinger 1978). In all cases, it was suggested that the large spatial dimensions and the high molecular weight of these molecules reduce membrane permeation rates and therefore the chemical uptake rate in the organism. Studies on membrane permeation rates of organic solutes in liposomes have demonstrated that membrane permeation rates are indeed inversely proportional to the molecular weight of the solute (Stein 1986).

However, Gobas et al. (1986) pointed out that for chemicals which are not metabolized, chemical uptake will involve the same transport processes as chemical elimination. In other words, if chemical uptake involves membrane permeation then chemical elimination involves permeation through the same membranes. If the size of the solute reduces membrane permeation it should affect both the uptake rate and elimination rate constants equally. Since the bioconcentration factor is the ratio of k_1 and k_2 it follows that bioconcentration factors are not affected by membrane permeation rates. The bioconcentration factor can often be viewed as a thermodynamic property, which reflects the solute's affinities for the lipid tissue and water. Provided exposure times are long enough bioconcentration factors are therefore inherently independent of kinetic factors.

The effect of membrane permeation rates on uptake and elimination kinetics in fish was also addressed by Gobas and Mackay (1987). It was shown that for high K_{ow} chemicals most of the resistance for uptake and elimination, and thus the rate determining step, lies in the aqueous phases of the fish and not in lipid phases such as biological membranes. Chemical transport in aqueous phases thus controls the uptake and elimination kinetics. This implies that for membrane permeation to affect uptake and elimination rates, the membrane resistance has to increase by several orders of magnitude. It thus seems inconceivable that the relatively small increase in molecular size, which is often associated with an increase in 1-octanol-water partition coefficient, could result in an increase of the membrane resistance large enough to affect the uptake and elimination kinetics.

In order to explain the complete lack of bioconcentration of octachloronaphthalene, hexabromobenzene and octachlorodibenzo-p-dioxin in the guppy (Poecilia reticulata) Opperhuizen et al. (1984, 1985) suggested that molecules with certain spatial dimensions are not able to cross the membrane-water interface and enter the membrane. It was hypothesized that membrane permeation, and thus bioconcentration, was blocked for molecules with minimal internal cross sections of 0.96 nm or larger. Bioconcentration experiments with hydrophobic dyes, which are molecules with very large molecular dimensions, support this hypothesis (Anliker and Moser 1987).

Gobas et al. (1989), however, observed high bioconcentration factors in the guppy for some brominated biphenyls with minimal internal cross sections similar to that of hexabromobenzene i.e.

0.96 nm. Muir et al. (1986) observed small amounts of octachlorodibenzo-p-dioxin and a metabolic product of octachlorodibenzo-p-dioxin in the rainbow trout and fathead minnow, which suggests uptake of octachlorodibenzo-p-dioxin in fish.

It can thus be concluded that from a mechanistic point of view it is conceivable that a "cut-off" diameter exists for passive membrane diffusion. However, the limited amount of data, the lack of consistent data and the confounding effects of other factors such as bioavailability make it difficult to determine if such a cut-off dimension exists and what its value is.

Exposure Time

It has been shown by several authors that the time needed to reach equilibrium between the water and the organism increases with increasing K_{ow}. This is the result of the slower elimination kinetics of high K_{ow} chemicals resulting in longer half-life times. Super-hydrophobic chemicals, with large log K_{ow} (i.e. larger than 5 to 6), thus require a very long time to reach equilibrium. However, bioconcentration factors are traditionally measured in relatively short experiments of 10 to 30 days. Measured bioconcentration factors of high K_{ow} chemicals are therefore often found to be lower than what they would have been in an experiment of longer duration. Hawker and Connell (1985) pointed out that this may lead to a curvi-linear relationship between K_c and K_{ow} and thus a loss of the linear relationship observed for chemicals of lower K_{ow}. To derive the real bioconcentration factor from such a bioconcentration experiment it is necessary to derive the rate constants k_1 and k_2 from uptake and elimination data. The bioconcentration factor can then be determined as k_1/k_2 and is independent of the actual exposure time.

The short duration of many bioconcentration experiments is an important factor contributing to the relatively low reported bioconcentration factors of high K_{ow} chemicals. However, it should be stressed that the these low bioconcentration factors are not real, but they are merely the result of experimental design and inaccurate treatment and interpretation of the bioconcentration data. The true bioconcentration factor is higher than the observed value and can simply be determined by using the appropriate data handling procedures. Some of these procedures are outlined by Gobas et al. (1989) and are discussed in detail in Chapter 8.

Reduced Bioavailability

Chemicals of low aqueous solubility have a high tendency to sorb onto, or aggregate with "dissolved" and particulate organic matter in the water. In bioconcentration experiments this often results in an apparent chemical concentration in the water, which is larger than the chemical concentration in true solution. Black and McCarthy (1988) showed that organic chemicals bound to dissolved organic matter are not able to diffuse through the gill membrane of fish. They are therefore not available for chemical uptake in organisms. This has been further supported by a number of studies in various systems using different aquatic organisms (Leversee et al. 1983, Spacie et al. 1983, Landrum et al. 1985, McCarthy and Jimenez 1985, McCarthy et al. 1985, Landrum et al. 1987).

Reduced bioavailability usually leads to an underestimate of the bioconcentration factor K_C (i.e. C_F/C_W) and uptake rate constant k_1, due to an overestimation of the bioavailable chemical concentration (Gobas et al. 1989). Measurements of the chemical concentration in the water are often not able to distinguish between truly dissolved and bound chemical. They thus reflect the total chemical concentration in the water, which is higher than the concentration of bioavailable chemical. Since the calculation of the bioconcentration factor and the uptake rate constant both require the input of the chemical concentration in the water, an overestimation of the bioavailable chemical concentration results in apparently low bioconcentration factors and uptake rate constants. If the bioavailable chemical concentration is determined (instead of the total chemical concentration) the real bioconcentration factor and uptake rate constant can be determined.

The fraction of chemical that is in true solution and thus available for uptake in the organisms is usually referred to as the available solute fraction ASF or bioavailability. It is the ratio of the truly dissolved chemical concentration in the water C_d and the total chemical concentration in the water C_t i.e.

$$ASF = C_d/C_t \tag{3.35}$$

Sorption of a chemical to dissolved organic matter in the water is generally viewed as a simple partitioning process of the chemical between the water and the dissolved organic matter (DOM):

Dissolved Chemical + DOM <==========> Solute-DOM

This partitioning process can be expressed by a partition coefficient K_p i.e.

$$K_p = C_b/(C_d.[DOM]) \tag{3.36}$$

where C_b is the concentration of bound or sorbed chemical i.e. $(C_t - C_d)$, C_d is the concentration of freely dissolved chemical and [DOM] is the concentration of dissolved organic matter in the water. Substitution of equation 3.36 in equation 3.35 results in the following equation for the available solute fraction in the water

$$ASF = 1/(1 + K_p.[DOM]) \tag{3.37}$$

Equation 3.37 demonstrates that the extent to which chemicals are available for chemical uptake in aquatic organisms is influenced by the concentration of DOM in the water and the chemical's partition coefficient K_p. For example, Black and McCarthy (1988) showed that at a similar DOM concentration of 3 mg carbon per liter water, benzo-a-pyrene, which has a 20 fold higher K_p than 2,2',5,5'-tetrachlorobiphenyl, was 7 times less bioavailable (i.e. lower ASF) than tetrachlorobiphenyl. McCarthy et al. (1985) showed a similar effect in Daphnia magna, where a DOM concentration of less than 1.5 mg carbon/l reduced the bioaccumulation of benzo-a-pyrene and benzanthracene in the daphnids. However, a much higher concentration of 60 mg carbon/l of DOM had little effect on the bioaccumulation of naphthalene, which has a lower affinity for binding to DOM and thus a lower K_p. Landrum et al. (1985) also showed that the availability of PAHs to Pontoporeia hoyi in the presence of Aldrich humic acids correlates well with the chemical's partition coefficient to DOM as well as to the concentration of DOM.

In chapter 2, which discusses the mechanism of chemical sorption, it has been shown that K_p can often be related to the chemical's hydrophobicity and 1-octanol-water partition coefficient. Chemicals with higher 1-octanol-water partition coefficients therefore, also have a higher K_p and thus a lower ASF. Although it is tempting to assume a relationship between the partition coefficient to DOM and the 1-octanol-water partition coefficient, experimental evidence supporting such a relationship is rare. For example, Black and McCarthy (1988) reported a 20 fold difference in K_p between benzo-a-pyrene and 2,2',5,5'-tetrachlorobiphenyl, whereas the 1-octanol-water partition coefficients of the these chemicals are approximately equal i.e. log K_{OW} are 6.4 and 6.1 respectively. Various studies showed that chemical partitioning to DOC does not completely reflect the 1-octanol-water partition coefficient and that K_p values can vary over several orders of magnitude depending on the nature or source of the DOM. (Landrum et al. (1984), Mccarthy and Jimenez (1985), Chiou et al. (1986), Means and Wijayaratne (1982), Evans (1988)).

It can thus be concluded, that presently there is little scientific basis for the prediction of the K_p of chemicals to naturally occurring DOM, and thus, the bioavailability of organic chemicals in natural waters. Studies into the mechanism of chemical binding to dissolved organic matter are needed, in order to develop predictive relationships for the bioavailability of organic chemicals in natural waters.

Chemical Elimination by Faecal Egestion

As discussed earlier in chapter 3.2, experimental evidence has shown that bioconcentration can often be viewed as a direct exchange of chemical between the water and the organism. A simple differential equation i.e. equation 3.2 gives the kinetic description of this process and assumes that chemical transfer is only across the respiratory surface of the organism (e.g. the gills for fish). However, fish and many other aquatic organisms eliminate absorbed chemicals in their urine and/or faeces. These routes of chemical elimination are usually ignored by bioaccumulation researchers. However, McKim et al. (1986) showed that significant amounts of chemical can be eliminated in the faeces.

If chemical elimination through faecal egestion follows first order kinetics, equation 3.2 should be extended to include the rate constant for chemical elimination into the faeces k_E to give

$$dC_F/dt = k_1.C_W - k_2.C_F - k_E.C_F \tag{3.38}$$

Since bioconcentration has been defined as the ratio of the chemical concentrations in the organism and water at steady state (i.e. dC_F/dt equals zero) it follows that the bioconcentration factor K_C for a non metabolizing chemical is

$$K_C = k_1/(k_2 + k_E) \tag{3.39}$$

Equation 3.39 demonstrates that when chemical elimination into the faeces is insignificant relative to elimination into the water (e.g. via the gills) i.e. $k_E \ll k_2$, K_C equals k_1/k_2. This ratio k_1/k_2 reflects the chemical's partition coefficient between the fish and the water, and therefore, corresponds with the chemical's 1-octanol-water partition coefficient. It thus follows that the relationship between log K_C and log K_{OW} is linear for chemicals exclusively eliminated to the water. However, when elimination into the faeces provides a significant route for chemical depuration, K_C equals $k_1/(k_2+k_E)$ and is therefore smaller than the fish-water partition coefficient k_1/k_2. In that case, K_C represents a balance of chemical uptake from the water and chemical elimination to both the water and the faeces. It is no longer an organism-water partition coefficient. Since the correlation between log K_C and log K_{OW} is based on the chemical's tendency to partition between the organism's lipids and water a linear correlation between log K_C and log K_{OW} will no longer exist. The bioconcentration factor will be lower than expected from the 1-octanol-water partition coefficient.

The extent to which chemical elimination lowers the bioconcentration factor depends on the relative rates of chemical elimination to the water and to the faeces. Gobas et al. (1989) established correlations for the elimination rate constants to the water (k_2) and to the faeces (k_E) in the guppy (Poecilia reticulata) with respect to the 1-octanol-water partition coefficient i.e.

$$1/k_2 = (2.5 \,.\, 10^{-5}.K_{OW}) + 3.0 \tag{3.40}$$

$$1/k_E = (1.6 \,.\, 10^{-6}.K_{OW}) + 68 \tag{3.41}$$

These correlations, which are also illustrated in Figure 3.4, allow the calculation of k_2 and k_E for organic chemicals, if K_{OW} is known. They demonstrate that for chemicals of relatively low K_{OW} i.e. log K_{OW} smaller than 6, k_2 is larger than k_E. The bioconcentration factor is therefore approximately k_1/k_2. A linear relationship between log K_C and log K_{OW} can be expected and is indeed often observed. However, for chemicals with a log K_{OW} larger than 6, chemical elimination into the faeces is the predominant route for chemical depuration (i.e. k_E is larger than k_2), thus causing a loss of linear relationship between log K_C and log K_{OW}. Although equations 3.40 and 3.41 apply to the guppy, it is expected that similar relationships can be found for other aquatic species.

It can thus be concluded that for chemicals with a log K_{OW} smaller than 6, bioconcentration is predominantly an exchange of chemical between the organism's lipid tissue and water. As a result a linear relationship can be expected between log K_C and log K_{OW}. For chemicals with log K_{OW} exceeding 6, bioconcentration is a balance between the rates of chemical uptake from the water and elimination to both the water and faeces. This transfer from a thermodynamically controlled fish-water partitioning process to a kinetically controlled balance between uptake from the water and

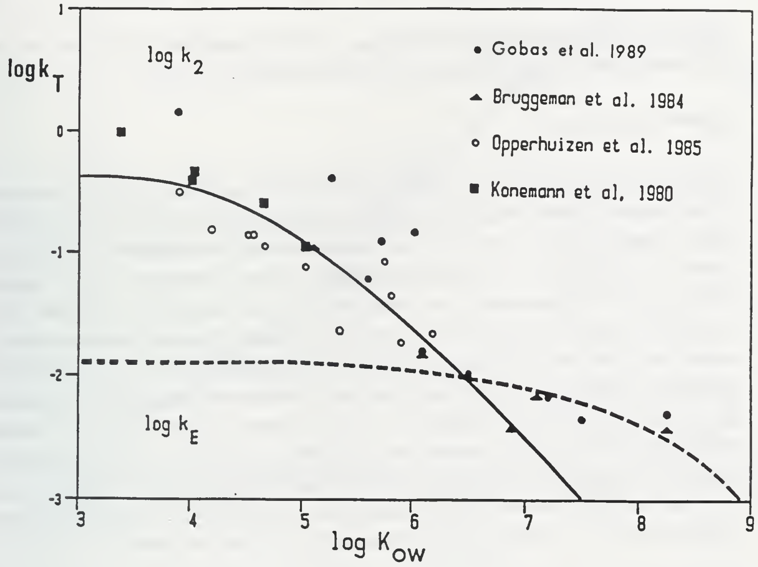


Figure 3.4

The logarithm of observed total elimination rate constants (log k_2) of selected aromatic hydrocarbons in the guppy (Poecilia reticulata) as a function of the logarithm of the 1-octanol-water partition coefficient. The solid line represents elimination of chemical to the water. The dotted line represents elimination into faecal matter.

elimination to the faeces with increasing K_{ow} contributes significantly to the loss of linear relationship between log K_c and log K_{ow} for chemicals of log K_{ow} above 6.

Lipid-Water Partitioning

As discussed earlier (chapter 3.4), the linear correlation between log K_c and log K_{ow} is the result of the similarity in phase properties between the organism's lipid tissue and 1-octanol towards organic chemicals. In other words, for many organic chemicals the solubilities in, or affinities for, 1-octanol and lipid tissue are approximately equal. 1-Octanol is therefore a suitable surrogate phase for lipids. The limitations of this simple comparison have rarely been investigated. Gobas et al. (1988) reported a study which compared the partitioning of organic chemicals between 1-octanol and water with that between biological membranes and water. An excellent linear correlation was found between the logarithms of the 1-octanol-water (K_{ow}) and membrane-water (K_M) partition coefficients for chemicals with a log K_{ow} between 1 and 6. However, this linear relationship did not extend to chemicals with log K_{ow} below 1 and above 6. For chemicals of log $K_{ow} > 6$, the membrane-water partition coefficient reached a maximum value of 10^6 for log K_{ow} of 7. It then tended to fall with further increasing K_{ow}. The observed relationship between the membrane-water and 1-octanol-water partition coefficient is shown in Figure 3.5.

The breakdown between membrane-water and 1-octanol-water partition coefficients is related to the membrane's and 1-octanol's ability to accomodate the solute. It was shown that with increasing volume of the solute, the membrane's capacity to store solutes drops more quickly than that of 1-octanol. This may be explained by the structured nature of biological membranes compared to the relatively unstructured "bulk-phase" nature of 1-octanol. Larger molecular size solutes may thus have greater difficulty dissolving in the rigid membrane matrix than in 1-octanol. Since membranes are an important component of the lipid tissue of any organism, it is possible that the 1-octanol-water partition coefficient overestimates the lipid-water partition coefficient for large molecular size chemicals. This may contribute to the discrepancy between 1-octanol-water and organism-water partition coefficients (or bioconcentration factors) for chemicals of high 1-octanol-water partition coefficients.

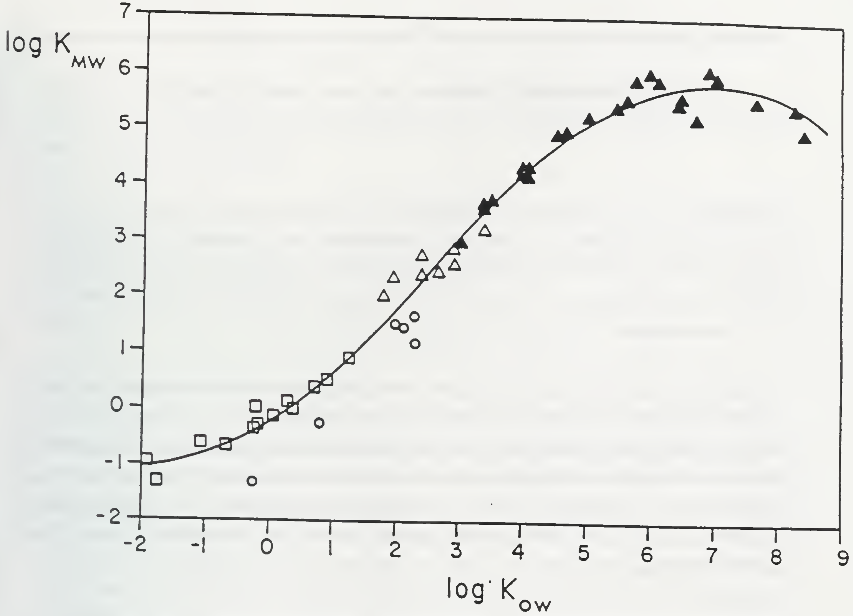


Figure 3.5

The logarithm of the partition coefficient between biological membranes and water (log K_M) versus the logarithm of the 1-octanol-water partition coefficient (log K_{OW}).

4. BIOCONCENTRATION KINETICS

4.1 Introduction

In chapter 3 it was shown that the mechanism underlying bioconcentration of organic chemicals in aquatic organisms is a thermodynamically controlled partition process between the lipids (i.e. biological membranes, micelles and other lipid storage sites in the organism) of the organism and the water. The extent to which bioconcentration occurs can be expressed by the ratio of chemical concentrations in the organism and water at steady-state or equilibrium. This ratio is generally referred to as the bioconcentration factor and can often be predicted from the 1-octanol-water partition coefficient.

This chapter reviews the kinetics of chemical uptake from the water and elimination to the water of organic chemicals. First, existing pharmacokinetic models for the bioconcentration process are discussed. Then, relationships between rate constants and the 1-octanol-water partition coefficient are reviewed and their predictive capabilities discussed. A detailed analysis of the mechanism of chemical uptake and elimination in fish is presented in section 4.4. This analysis results in a model for the prediction of chemical uptake and elimination kinetics in various aquatic organisms such as fish and benthic inverterbrates. Finally, factors affecting chemical uptake and elimination kinetics in aquatic organisms are discussed is section 4.5.

4.2 Kinetic Bioconcentration Models for Aquatic Organisms

From a physiological point of view, organisms can be considered as a collection of compartments, each providing a storage site for chemicals, accessed at a specific rate. However, when quantifying simple chemical uptake and elimination kinetics, organisms are usually treated either as a single compartment or a combination of two compartments. In most cases, experimental detail is insufficient to distinguish between more than one or two compartments in the organism. As a result one or two compartment kinetic models will be considered to describe the kinetics of chemical uptake and elimination.

The one-compartment-organism model (i.e. organism-water) was discussed earlier in chapter 3. It assumes that the organism is one homogeneous compartment, in which all chemical is equally accessible to uptake and depuration. The general acceptability of this model has been demonstrated in a large number of experimental studies. It can therefore be considered to give a reliable description of the bioconcentration of organic chemicals in most aquatic organisms.

The Two-Compartment-Organism Model

Although most experimental data are adequately described by the one-compartment-organism model, some studies have shown that a two-compartment-organism model gives a more accurate picture of the bioconcentration kinetics (Konemann & Van Leeuwen 1980, Gooch and Hamdy 1982). Such models regard the organism as having two compartments (e.g. two lipid pools) which are accessed at two distinct rates. For example, a substance may bioconcentrate in a compartment A (e.g. blood) of an organism at a rate of 1 mol/day but in the rest of the fish, i.e. compartment B, at a much slower rate of 0.1 mol/day. Compartment A will therefore "fill up" more quickly than compartment B. When the chemical is being eliminated, it will eliminate from compartment A more rapidly than from compartment B.

The two-compartment-organism model distinguishes between three compartments in total i.e. the water, organism compartment A of volume V_{F1} and organism compartment B of volume V_{F2}. Compartment B is accessed through compartment A. Chemical transfer is therefore between the water and compartment A and between compartments A and B (and not between the water and compartment B). The rate constants for chemical transport are assumed to be first order. A schematic diagram of the two-compartment-organism model is presented in Figure 4.1.

The following differential equations describe the kinetics of the two compartment organism model:

$$dC_W/dt = 0 \tag{4.1}$$

i.e. when the chemical concentration in the water is constant

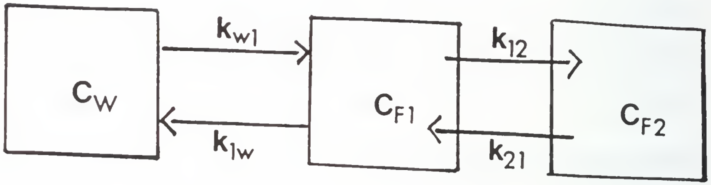


Figure 4.1

Schematic diagram of a two-compartment-organism model.

$$dC_{F1}/dt = k_{W1}.C_W + k_{21}.C_{F2} - k_{12}.C_{F1} - k_{1W}.C_{F1} \tag{4.2}$$

$$dC_{F2}/dt = k_{12}.C_{F1} - k_{21}.C_{F2} \tag{4.3}$$

where k_{W1} and k_{1W} are the first order rate constants of chemical transfer between respectively the water and compartment A, and compartment A and the water. The rate constants k_{12} and k_{21} refer to chemical transport within the organism, namely between the compartments A and B. The total chemical concentration in the organism C_F can be expressed as the sum of the concentrations in the compartments A and B as

$$C_F = (C_{F1}.V_{F1} + C_{F2}.V_{F2})/(V_{F1} + V_{F2}) \tag{4.4}$$

After (i) integration of the differential equations 4.2 and 4.3 with a constant concentration in the water and (ii) substitution of C_{F1} and C_{F2} in equation 4.4 it follows that

$$C_F = A.C_W.(1 - \exp[-a.t]) + B.C_W.(1 - \exp[-b.t]) \tag{4.5}$$

Equation 4.5 demonstrates that at steady state, which corresponds with an infinite exposure time t, the concentration in the organism will be $(A + B)$ where $(A + B)$ equals

$$(A + B) = \{(k_{W1}/k_{1W}) + (k_{W1}.k_{12}/k_{1W}.k_{21})\}.C_W \tag{4.6}$$

The bioconcentration factor i.e. the ratio of chemical concentrations in fish and water at steady state is therefore

$$K_C = (A + B)/C_W = (k_{W1}/k_{1W}) + (k_{W1}.k_{12}/k_{1W}.k_{21}) \tag{4.7}$$

Expressions for a and b and the rate constants k_{W1}, k_{1W}, k_{12} and k_{21} can be obtained by solving the second order differential equations in C_{F1} and C_{F2}. This procedure has been described by Konemann and Van Leeuwen (1980) and Gibaldi and Perrier (1982) and only the results are given here

$$a.b = k_{1W}.k_{21} \tag{4.8}$$

$$a + b = k_{1W} + k_{12} + k_{21} \tag{4.9}$$

$$k_{21} = (A.b + B.a)/(A + B) \tag{4.10}$$

If A, B, a and b have been determined from experimental data k_{21} can be calculated from equation 4.10. Then, k_{1W} can be determined from equation 4.8 as

$$k_{1W} = a.b/k_{21} \tag{4.11}$$

and k_{12} follows from equation 4.9, i.e.

$$k_{12} = a + b - k_{21} - k_{1W} \tag{4.12}$$

The rate constant h_{W1} then follows from equation 4.7.

Integration of equations 4.2 and 4.3, for depuration conditions in which the concentration in the water is zero gives the following expression for C_F

$$C_F = A^*.\exp(-a.t) + B^*.\exp(-b.t) \tag{4.13}$$

where A^* and B^* are constants relating to the concentration in the organism at the start of the elimination period, i.e. $C_{F,0}$, as

$$(A^* + B^*) = C_{F,0} \tag{4.14}$$

When at the start of the elimination period the organism is at steady state it follows that A^* equals A and B^* is B.

The variables A,B,a and b can be derived from uptake and elimination data. Usually they are calculated from elimination data. A typical plot for chemical elimination to the water following the two compartment organism model is presented in Figure 4.2. The initial phase represents the relatively rapid elimination from compartment A. The later phase represents chemical transfer from compartment B to A. The sum of A^* and B^* i.e. $(A^* + B^*)$ represents the initial concentration in the organism. The intercept which follows from the linear regression of the later part of the ln C_F versus t curve is B. The constant A follows from the slope of the ln C_F versus t curve at the initial time (i.e. t is zero). Often it can be determined as the slope of the linear regression of the initial ln C_F versus time data. The constant b can be calculated as the slope of the linear regression of the ln C_F versus time data of the later part of the elimination period.

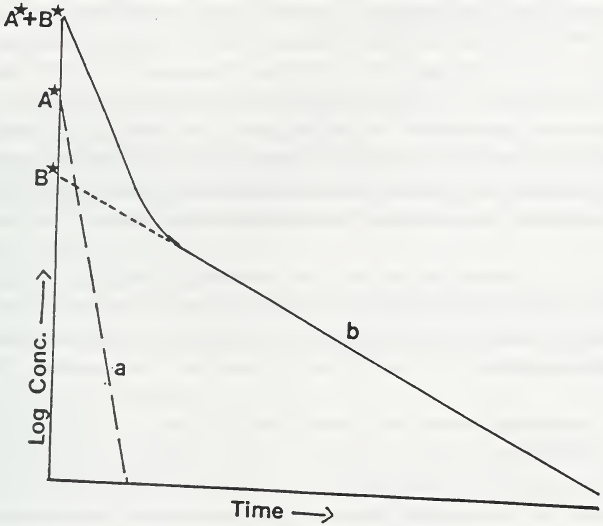


Figure 4.2

Chemical elimination following a two compartment organism model with first order rate constants.

Konemann and Van Leeuwen (1980) showed that the bioconcentration kinetics of chlorobenzenes in guppies (Poecilia reticulata) can be described by a two-compartment-organism model. Gooch and Hamdy (1982) reported similar observations for PCB in guppies and Cichlids (*Cichlasoma facetum*). However, Bruggeman et al. (1984) and Opperhuizen et al. (1985) showed that the elimination kinetics of chlorobenzenes and PCBs in the guppy are consistent with a one-compartment-organism model. This lack of agreement may be due to experimental error in the measurement of organism concentrations, for example as a result of individual variations between the test organisms. The presence of experimental error makes it often difficult to observe relatively subtle differences between initial and later elimination rates.

In general, it is concluded that the use of a two compartment model is only justified if the data are of high quality and show distinct non-linearity. It is possible that with improved experimental methodology the multi-compartmental nature of chemical uptake and depuration dynamics will become more evident.

4.3 Relationships Between Bioconcentration Rate Constants and the 1-Octanol-Water Partition Coefficient

Studies on the bioconcentration kinetics of organic chemicals have shown that the rate constants of chemical uptake (k_1) and elimination (k_2) can often be related to the 1-octanol-water partition coefficient (K_{OW}). These relationships are of great value because they provide a method to estimate the bioconcentration rate constants for other chemicals from measurements of the 1-octanol-water partition coefficient.

Elimination rate constants (k_2) tend to decrease with increasing K_{OW}. Chemicals of high K_{OW} are therefore eliminated by organisms to the water more slowly and have longer half lives than chemicals of lower K_{OW}. Various empirical relationships between the elimination rate constant and the 1-octanol-water partition coefficient have been reported. For example, Konemann and Van Leeuwen (1980) showed that for chlorobenzenes in guppies (Poecilia reticulata) the logarithms of the elimination rate constants and the 1-octanol-water partition coefficient follow a linear relationship i.e.

$$\log k_2 = - 0.419 . \log K_{OW} + 1.435 \qquad (4.15)$$

The behaviour of the uptake rate constant with the 1-octanol-water partition coefficient is less obvious. For example, Konemann and Van Leeuwen (1980) showed a linear correlation between log k_1 and log K_{OW}, i.e.

$$\log k_1 = 0.462 . \log K_{OW} + 1.837 \tag{4.16}$$

Opperhuizen et al. (1985), however, found that the uptake rate constants of polychlorinated naphthalenes, chlorobenzenes, PCBs and other chemicals in the guppy are independent of the 1-octanol-water partition coefficient of the chemical. For all chemicals a similar uptake rate constant of approximately 1000 d^{-1} was determined.

Bruggeman et al. (1981) observed that the uptake rate constants of PCBs in goldfish (Carassius auratus) decreased with increasing K_{OW}. Muir et al. (1986) proposed a parabolic relationship with log K_{OW} with an optimum uptake rate constant for chemicals with a log K_{OW} of approximately 6 to express the relationship between log k_1 and log K_{OW}.

To explain the observed relationships between the uptake and elimination rate constants and the 1-octanol-water partition coefficient, various authors have addressed the mechanism of chemical uptake and elimination. These studies are now reviewed.

4.4 Models for the Bioconcentration Kinetics in Aquatic Organisms

To be absorbed and bioconcentrated in the lipid tissue of an organism, molecules have to move from the ambient water through the gill membrane(s), into the blood stream and possibly through other membranes to their "final" storage site. During this process these molecules are associated with, transferred by, and transported through numerous phases of different nature. For example, gill ventilation involves transport of a chemical in the water by a flow process, membrane transfer is a diffusion of chemical through the lipid portion of the membrane. In the blood stream, the chemical is associated with lipoid matter and blood proteins moving at the blood flow rate. Each of these phases provides a certain "conductivity" or its reciprocal, a "resistance" to chemical transport and thus affects the rate at which the chemical bioconcentrates in the organism. Various authors have studied and modelled the dynamics of chemical transfer in aquatic organisms in terms of phase resistances. This has resulted in two models i.e. the membrane-permeation model and the lipid-water mass transfer model.

Table 4.1

Reported linear correlations between the logarithm of the uptake (log k_1) and the elimination (log k_2) rate constants and the logarithm of the 1-octanol-water partition coefficient (log K_{ow}).

SPECIES	CORRELATION	n	r²	REFERENCE
Guppies	log k2 = -0.419 .log Kow + 1.435	6	0.98	Konemann & Van Leeuwen 1980
Guppies & Rainbow trout	log k2 = -0.414 .log Kow + 1.47	14	0.9	Spacie & Hamelink 1982
Goldfish	log k2 = -0.891 .log Kow + 2.182		0.95	Hawker and Connell 1985
Rainbow trout	log k2 = -0.563 .log Kow + 0.587		0.88	Hawker and Connell 1985
Various	log k2 = -0.663 .log Kow + 0.947		0.95	Hawker and Connell 1985
Molluscs	log k2 = - 0.54 .log Kow + 0.983	6		Hawker & Connell 1986
Daphnids (Daphnia Pulex)	log k2 = -0.507 .log Kow + 2.053	11		Hawker & Connell 1986
Guppies	log k1 = 0.462 .log Kow + 1.837	6	0.86	Konemann & Van Leeuwen 1980
Guppies & Rainbow trout	log k1 = 0.147 .log Kow + 1.98	14	0.55	Spacie & Hamelink 1982
Guppiea	log k1 = 0.429 .log Kow + 0.418	10	0.85	Saarikoski et al. 1986
Daphnids (Daphnia Pulex)	log k1 = 0.391 .log Kow + 0.738	11		Hawker & Connell 1986
Molluscs	log k1 = 0.304 .log Kow - 0.252	7		Hawker & Connell 1986

Membrane-Permeation Model

The membrane-permeation model draws on the work of Zwolinski et al. (1949), Stehle and Higuchi (1972), Ho et al. (1977) and Flynn and Yalkowsky (1972) on the permeation of non-electrolytes through membranes. These authors have described the permeation of organic molecules through biological membranes as a passive diffusion process through the phospholipid bilayer and aqueous diffusion layers associated with the membrane. In this approach, membrane permeation is viewed as the result of two independent diffusion processes i.e. in aqueous diffusion layers and in the lipid bilayer, which apply in series. The total resistance of membrane permeation R_T can therefore be expressed as the sum of the resistances in the aqueous diffusion layers R_D and in the lipid membrane R_M (Flynn and Yalkowsky 1972) i.e.

$$R_T = R_D + (R_M/K_M) \qquad (4.17)$$

where K_M is the membrane/water partition coefficient. K_M is introduced into equation 4.17 to correct for the differences in chemical concentrations in the water and the lipid membrane. Yalkowsky and Morozowich (1980) extended this model to account for the unusually high permeation rates of low molecular weight polar compounds by adding a third term i.e R_P for chemical diffusion through aqueous pores in the membranes:

$$R_T = R_D + (R_M/K_M) + R_P \qquad (4.18)$$

The flux of chemical through the biological membrane F_T (i.e. the amount of chemical transported per unit of time) can be expressed as

$$F_T = (1/R_T).A.dC \qquad (4.19)$$

where A is the diffusion area and dC is the concentration gradient across the complete membrane. Substitution of equation 4.18 in equation 4.19 results in

$$F_T = A.dC/(R_D + (R_M/K_M) + R_P) \qquad (4.20)$$

Membrane permeation of low molecular weight chemicals (MW < 50 g/mole) is predominantly through the pores, with virtually no diffusion through the lipid membrane. These chemicals are usually highly water-soluble, with low K_{ow} values and therefore of little interest from a bioconcentration viewpoint.

For chemicals that are less soluble or are too large to move through the pores, R_P can be removed from equation 4.20 (or equation 4.17 used) such that

$$F_T = A.dC/(R_D + (R_M/K_M)) \tag{4.21}$$

For slightly hydrophobic chemicals with moderate values of K_M, the term R_M/K_M is greater than R_D. Their rate of permeation through the lipid membrane approaches $A.dC.K_M/R_M$, and increases with increasing K_M, assuming that R_M is approximately constant. This results in an increase of the total flux through the membrane, which is directly related to the increase in membrane/water partition coefficient of the chemical. For these chemicals, membrane permeation is controlled by diffusion in the membrane.

However, for more hydrophobic chemicals, with large K_M and small R_M/K_M, the primary chemical resistance lies in the aqueous diffusion layers, i.e. R_D. Aqueous diffusion becomes more important and eventually controls the flux. For very high K_M chemical diffusion through aqueous diffusion layers controls the flux. The total flux no longer depends on K_M, approaching AdC/R_D.

Since membrane-water partition coefficients are often directly related to the 1-octanol-water partition coefficient (Gobas et al. 1988), the membrane permeation rates of organic chemicals can be classified based on the 1-octanol-water partition coefficient as follows:

Low K_{ow}: i.e. $K_{ow} \ll R_M/R_D$	Low membrane permeation rate Diffusion is through aqueous pores No relationship with K_{ow}
Medium K_{ow}: i.e. $K_{ow} = R_M/R_D$	Diffusion through the lipid membrane controls the flux and permeation rate The membrane permeation rate and flux are near linearly dependent on K_{ow}
High K_{ow}: i.e. $K_{ow} \gg R_M/R_D$	Diffusion through aqueous diffusion layers controls the total membrane permeation rate and flux Membrane permeation rate is not dependent on K_{ow}

This "two resistance in series" concept is further illustrated in Figure 4.3 and is supported by a number of experimental studies (Yalkowsky et al. 1974).

Several authors have applied this membrane permeation model to the bioconcentration kinetics of organic chemicals in aquatic organisms. The inherent assumptions in this approach are that:

(i) organic chemicals are absorbed and bioconcentrated by passive diffusion across the respiratory surface

(ii) membrane-permeation across the respiratory surface is the rate determining step for bioconcentration in organisms

McKim et al. (1985) applied the model for the chemical flux across membranes i.e. equation 4.20 to explain the observed relationship between the gill uptake efficiency in rainbow trout of 14 organic chemicals and the 1-octanol-water partition coefficient. These authors measured the uptake efficiencies of 14 organic chemicals, varying in log K_{ow} from 0.23 to 7.5, in rainbow trout by monitoring the inspired and expired water. They found that for chemicals with log $K_{ow} < 1.0$ the uptake efficiency was approximately 7% and did not vary with K_{ow}. For chemicals with log K_{ow} between 1 and 3 the uptake efficiency increased linearly with increasing K_{ow} i.e. from 7% to 60%. For chemicals with log K_{ow} from 3 to 6 they observed a constant uptake efficiency of 60%. To illustrate the relationship between the uptake efficiency and K_{ow} the data of this study have been replotted in Figure 4.4. Based on the similarity between relationships of membrane permeation and gill uptake efficiency with K_{ow} the authors concluded that membrane diffusion controls the uptake kinetics in rainbow trout. The authors, however, did not establish a direct relationship between the chemical flux through membranes and the gill uptake efficiency. The only observation which was not explained by the membrane permeation model was the drop in uptake efficiency for chemicals of very high log K_{ow} i.e. larger than 6. The high molecular weights and volumes of these high K_{ow} chemicals and self-association of the chemical were suggested as possible causes for this phenomenon.

Spacie and Hamelink (1982) proposed the membrane permeation model as a possible explanation for the observed loss of linear correlation between the bioconcentration factor and the 1-octanol-water partition coefficient. However, the authors did not elaborate on how the kinetic expressions for chemical transfer through membranes account for changes in equilibrium levels between water and organism expressed by the bioconcentration factor.

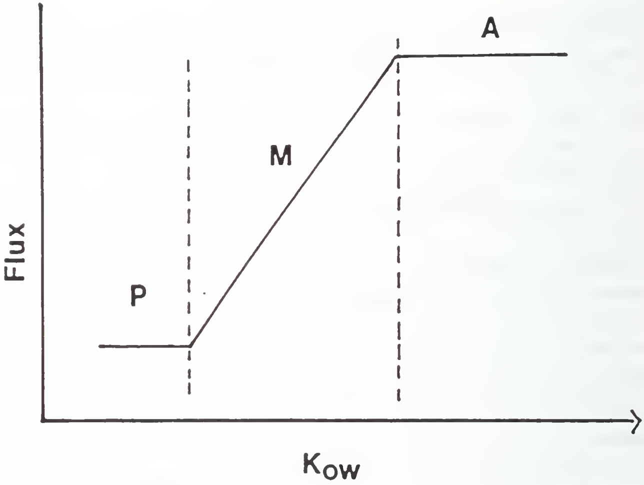


Figure 4.3

Flux through a biological membrane as a function of the 1-octanol-water partition coefficient illustrating the control of flux by pore diffusion (P), membrane diffusion (M) and diffusion through aqueous diffusion layers (A).

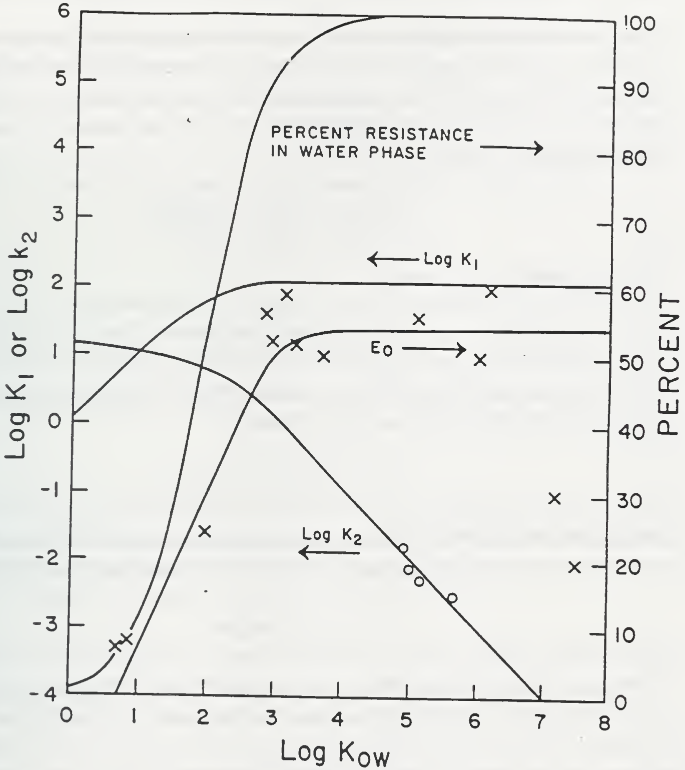


Figure 4.4

Gill uptake efficiencies (E_0) of various organic substances in the rainbow trout versus the 1-octanol-water partition coefficient (i.e. crosses (X) represent data points).

A link between membrane permeation and bioconcentration kinetics was established by Gobas et al. (1986). The authors proposed a kinetic bioconcentration model for fish, in which uptake and elimination are considered to be controlled by diffusion through membranes in the organism. The total flux of chemical into the fish i.e. $V_F.dC_F/dt$ or $k_1.V_F.C_W$ was expressed in terms of a flux through membranes i.e. $A.C_W/R_T$ such that

$$V_F.dC_F/dt = k_1.V_F.C_W = A.C_W/R_T \tag{4.22}$$

Rearranging equation 4.22 results in

$$k_1 \;.\; A/(V_F.R_T) \tag{4.23}$$

After substitution of equation 4.17 into equation 4.23 the following relationship for the bioconcentration uptake rate constant k_1 was obtained

$$1/k_1 = (V_F/A).\{R_D + (R_M/K_M)\} \tag{4.24}$$

This equation shows that for chemicals, for which membrane permeation is controlled by diffusion in the lipid membrane (low K_M and $R_M/K_M > R_D$), k_1 will be a linear function of K_M (or K_oW) i.e.

$$k_1 = (A/V_F.R_M).K_M \tag{4.25}$$

If diffusion in aqueous diffusion layers controls membrane permeation, such as for high K_M chemicals (i.e. $R_D > R_M/K_M$), k_1 will be constant and independent of K_M (or K_{OW}) i.e.

$$k_1 = A/V_F.R_D \tag{4.26}$$

Similarly, expressions for the elimination rate constant were derived by expressing the total flux of chemical from the organism to the water as a flux across the membranes i.e.

$$V_F.dC_F/dt = -k_2.V_F.C_F = -k_2.V_F.L_F.C_L = -A.C_{WF}/R_T \tag{4.27}$$

where C_{WF} is the chemical concentration in the water compartment of the organism at the inside of the membrane. Since chemical in this water compartment is assumed to be in equilibrium with that in the lipids it follows that the lipid-water partition coefficient K_L is C_L/C_{WF}, which after substitution in equation 4.27 gives

$$1/k_2 = V_L.K_L.R_T/A \tag{4.28}$$

Substitution of equation 4.16 in equation 4.28 and assuming that K_L equals K_M gives

$$1/k_2 = (V_L/A).(R_D.K_M + R_M) \tag{4.29}$$

Equation 4.29 demonstrates that when diffusion in the lipid membrane controls the elimination kinetics (typically, for low K_M chemicals) k_2 equals $A/V_L.R_M$ and is constant, whereas for chemicals for which membrane permeation is controlled by diffusion in aqueous layers (typically, for high K_M or K_{OW} chemicals) k_2 will decrease with increasing K_M (or K_{OW}) according to

$$k_2 = A/V_L.R_D.K_M \tag{4.30}$$

It was observed that, in agreement with the model prediction, the uptake rate constants of chlorobenzenes and chloronaphthalenes in the guppy increased with K_{OW} for chemicals of log K_{OW} below approximately 4.0 and was constant for chemicals of higher K_{OW}. The elimination rate constant was observed to decrease with increasing K_{OW} for the high K_{OW} chemicals.

Barber et al. (1988) also used the membrane permeation model to describe chemical transport across the gill membrane in a bioenergetic based kinetic bioconcentration model.

The strength of the membrane permeation models is that it provides a qualitative explanation of the observed behaviour of the bioconcentration kinetics, based on a sound and well established understanding of membrane permeation. However, the inherent assumptions that (i) organic chemicals are absorbed and bioconcentrated by passive diffusion across the respiratory surface and that (ii) membrane-permeation across the respiratory surface is the rate determining step for bioconcentration in organisms have not been adequately tested.

Lipid-Water Mass Transfer Model

Lipid-water mass transfer models were derived by Gobas and Mackay (1987) and Mackay and Hughes (1984) to gain further insight into the processes controlling the exchange of chemical between aquatic organisms and water and to develop practical procedures to estimate the bioconcentration kinetics of chemicals in fish. The authors used the fugacity approach to derive the model equations but presented their final equations in terms of rate constants. This model is an alternative of the two resistance model described earlier but it is written in fugacity terms.

The main feature of this model is that it views the exchange of dissolved chemical between the water and the organism to take place in a series of aqueous and lipid layers. All transport processes in water phases are grouped together in one overall water phase transport parameter D_W. This overall water phase transport parameter contains all transport parameters $D_{W,i}$ in water phases. The transport parameters $D_{W,i}$ can refer to diffusion, in which case $D_{W,i}$ equals $k.A.Z_W$, where k is the mass transfer coefficient (m/s), A is area of diffusion and Z_W is the chemical's fugacity capacity in the water phase. It can also refer to non-diffusive transport, where the solute is conveyed by a fluid flow G (m^3/s) such that $D_{W,i}$ equals $G.Z_W$. The overall transport parameter D_W can also be expressed as $Q_W.Z_W$, where the transport parameter Q_W (m^3/s) combines all k.A and flow rates G in water phases of the organism. The transport parameter Q_W can be viewed as a "hypothetical water flow rate" in the organism.

Similar to D_W, all transport processes in lipid phases are combined in an overall lipid phase transport parameter D_L. This lipid phase parameter D_L can be designated $Q_L.Z_L$, where Q_L combines all k.A and flow rates G in lipid phases of the organism and can be considered the "hypothetical lipid flow rate" in the organism. To simplify the transport parameters D_W and D_L, refer to the net transport of chemical in the water and the lipid phase of the organism. Assuming that the water and lipid transport processes apply in series, it follows that the transport parameter for chemical exchange between the water and the organism D_F can be expressed as

$$1/D_F = 1/D_W + 1/D_L = 1/Q_W.Z_W + 1/Q_L.Z_L \qquad (4.31)$$

In section 3 it was shown that D_F equals $k_1.V_F.Z_W$ or $k_2.V_F.Z_F$ and Z_F equals $L_F.Z_L$ and L_F equals V_L/V_F equation 4.31 can be rewritten as

$$1/k_2 = V_L.\{(Z_L/Q_W.Z_W) + 1/Q_L\} \tag{4.32}$$
$$1/k_1 = V_L.\{1/Q_W + (Z_W/Q_L.Z_L)\}/L_F \tag{4.33}$$

When the lipid-water partition coefficient Z_L/Z_W or K_L is replaced by the 1-octanol-water partition coefficient Z_O/Z_W or K_{OW} (thus assuming Z_L to be equal to Z_O) equations 4.32 and 4.33 become

$$1/k_2 = V_L.\{(K_{OW}/Q_W) + 1/Q_L\} \tag{4.34}$$
$$1/k_1 = V_L.\{1/Q_W + (1/Q_L.K_{OW})\}/L_F \tag{4.35}$$

The ratios V_L/Q_L and V_L/Q_W are the times required for transport of chemical in V_L m^3 of respectively lipids and water. If transport of a given amount of chemical requires a volume V of lipid, it will require a much larger volume i.e. $K_{OW}.V_L$ of water, since the chemical concentration in the water is a factor K_{OW} lower than that in the lipids. The time for the water phase in the organism to transport a certain amount of chemical is therefore K_{OW} times longer than that for the lipid phase. The transport time of the water phase is therefore multiplied with K_{OW} in equation 4.35, or alternatively the lipid transport time is divided by K_{OW} in equation 4.34. Since the lipid and water transport processes occur in series these times are additive and the longer time "controls" the bioconcentration kinetics. These "times" are essentially "resistances" with long time reflecting high resistance and slow transfer.

A similar equation was derived for the gill uptake efficiency in fish. The gill uptake efficiency, E, is defined as the ratio of the flux of chemical into the fish (i.e., $D_F.(f_W-f_F)$) to the flux of chemical into the gill compartment (i.e., $D_V.f_W$), namely

$$E = (D_F/D_V).\{(f_W - f_F)/f_W\} \tag{4.36}$$

where D_F is the net transport parameter for chemical uptake in the fish. D_V is the transport parameter for chemical transport through the gill compartment and is the product of the volumetric ventilation rate G_V (i.e. in m^3/s) and Z_W i.e. $G_V.Z_W$. At the beginning of an uptake period, when no compound is present in the fish and f_F is zero, the initial uptake efficiency E_0 is maximal i.e.

$$E_0 = D_F/D_V \tag{4.37}$$

When equation 3.14 i.e.

$$f_F = f_W.\{1 - \exp(-D_F.t/V_F.Z_F)\} \tag{4.38}$$

is substituted in equation 4.37, an expression for the time dependence of the gill uptake efficiency is derived, namely

$$E = (D_F/D_V).\exp(-D_F.t/V_F.Z_F) \tag{4.39}$$

which shows that the uptake efficiency decreases exponentially with time and will eventually reach zero as the fish reaches equilibrium. When equation 4.31 is substituted in equation 4.37 and Z_L equals Z_O and K_L equals K_{OW} the following equation for the initial or maximal uptake efficiency E_0 is obtained:

$$1/E_0 = D_V/D_F = G_V.\{1/Q_W + 1/(Q_L.K_{OW})\} \tag{4.40}$$

Equation 4.40 shows that the gill uptake efficiency is related to Q_W and Q_L in a similar fashion to that of the uptake rate constant. From equations 4.35 and 4.40 it follows that k_1 and E_0 are related by

$$k_1 = E_0.G_V/V_F \tag{4.41}$$

In summary, Gobas and Mackay (1987) derived three kinetic expressions describing chemical uptake and elimination in fish i.e.

$$1/k_2 = V_L.\{(K_{OW}/Q_W) + 1/Q_L\} \tag{4.42}$$
$$1/k_1 = V_L.\{1/Q_W + (1/Q_L.K_{OW})\}/L_F \tag{4.43}$$
$$1/E_0 = D_V/D_F = G_V.\{1/Q_W + 1/(Q_L.K_{OW})\} \tag{4.44}$$

These expressions contain parameters i.e. V_L, Q_W, Q_L, G_V and L_F, which are specific to a particular fish and its physiological condition and a chemical parameter K_{OW} expressing the chemical's tendency to partition between lipids and water.

When, for a series of chemicals varying in K_{OW}, rate constants or uptake efficiencies have been determined in a particular organism, equations 4.42 to 4.44 can be fitted to the experimental data resulting in values for Q_W and Q_L. The data fitting involves a regression e.g. $1/E_0$ versus $1/K_{OW}$ or

$1/k_2$ versus K_{OW}. Since K_{OW} usually varies over several orders of magnitude and therefore, weighs heavily in favour of the data points with high K_{OW}, simple linear regression (e.g. $1/E_0$ versus $1/K_{OW}$) leads to erroneous results. Instead, regression should be performed on a semi-logarithmic basis e.g. $1/E$ versus log K_{OW} and $1/k_2$ versus log K_{OW} but using the linear equations 4.42 to 4.44. Fitting these equations to experimental data is best performed using non-linear regression.

Gobas and Mackay (1987) fitted equations 4.42 to 4.44 successfully to kinetic data for the rainbow trout, goldfish and guppy, resulting in values for Q_W and Q_L. The fitted values for Q_W and Q_L are presented in Table 4.2. For example, based on the uptake efficiency data reported by McKim et al. (1985) in rainbow trout, with an average weight of 750 gram and lipid content of 9.8%, the authors found the following relationships for $1/E_0$

$$1/E_0 = 1.81 + 142/K_{OW} \tag{4.45}$$

When Q_W and Q_L are substituted in equations 4.42 and 4.43 expressions are obtained for k_1 and k_2 i.e.

$$1/k_1 = 0.0080 + 0.63/K_{OW} \tag{4.46}$$

$$1/k_2 = 0.00087.K_{OW} + 0.068 \tag{4.47}$$

Figure 4.4 further illustrates the satisfactory fit of the uptake efficiency data by equations 4.42 to 4.44.

Equations 4.42 to 4.44 give a quantitative picture of the relative contributions of the water and lipid phase transport to the bioconcentration kinetics. This is further illustrated in Table 4.3, where the water and lipid phase resistances are listed for chemicals of varying K_{OW}. It can be observed that for compounds of low K_{OW} i.e. smaller than 10, the bioconcentration kinetics in the rainbow trout is primarily controlled by transport processes in the lipid phases of the fish (i.e. $1/Q_L > K_{OW}/Q_L$). As a result, the uptake efficiency and uptake rate constant increases with increasing K_{OW} and the elimination rate constant is independent of K_{OW}. However, when K_{OW} increases, the amount of chemical that can be transported in the water phase decreases with respect to that in the lipid phase and the water phase resistance increases relative to the lipid phase resistance. This demonstrates that for chemicals of a K_{OW} of 1,000 or higher, the kinetics are primarily controlled by transport in aqueous phases of the rainbow trout (i.e. $K_{OW}/Q_W > 1/Q_L$) and the uptake efficiency and uptake

Table 4.2

Observed and calculated transport parameters for hydrophobic organic chemicals in different fish species showing relationships of Q_W and Q_L to body weight M.

Fish species	$\bar{M}$ (g)	V_L (L)	Q_W (L/day)	Q_L (L/day)	G_V(calc)
rainbow trout	750	82×10^{-3}	94	1.2	143
rainbow trout	900	98×10^{-3}	89	0.98	165
goldfish	4.5	0.18×10^{-3}	5.4		2.4
guppy	0.62	0.037×10^{-3}	0.51		0.49
guppy	0.35	0.026×10^{-3}	1.0		0.31

rate constants are independent of the K_{ow} and that the elimination rate constant decreases with increasing K_{ow}. This also demonstrates that for chemicals of high K_{ow} membrane permeation, which is a transport process in a lipid phase, is relatively unimportant compared to chemical transport in water phases. Membrane permeation is therefore not a rate determining step and does not have a major influence on the uptake and elimination kinetics of organics in fish.

The authors also investigated if the transport parameters Q_W and Q_L of chemical uptake also apply to chemical elimination. The transport parameters, deduced from uptake efficiency data (McKim et al. 1985), were compared to those derived from independently determined elimination rate constants in rainbow trout of approximately the same size (Niimi and Oliver 1983). The excellent agreement between the transport parameters derived from uptake and elimination experiment indicates that the transport parameters Q_W and Q_L are indeed similar for uptake and elimination.

4.5 Factors Affecting Uptake and Elimination Rate Constants

It was demonstrated in the previous section that the chemical's hydrophobicity (usually expressed by the 1-octanol-water partition coefficient) is an important factor controlling the uptake and elimination kinetics in aquatic organisms. Three other factors have been reported to affect the uptake and elimination rate constants. These are (i) the size or body weight of the organism, (ii) the oxygen concentration in the water and (iii) temperature. These factors are now discussed.

Body Weight of the Organism

Gobas and Mackay (1987) derived values for Q_W and Q_L for different fish species from experimental data. They showed that Q_W varies from fish to fish but tends to increase with increasing fish weight M. It was shown that Q_W is proportional to M raised to a power of 0.6 (+/- 0.2), namely

$$Q_W = 1.4.M^{0.6} \tag{4.48}$$

This relationship is illustrated in Figure 4.5. Although conclusive data are lacking, it is believed that Q_L follows a similar relationship i.e.

$$Q_L = 0.014.M^{0.6} \tag{4.49}$$

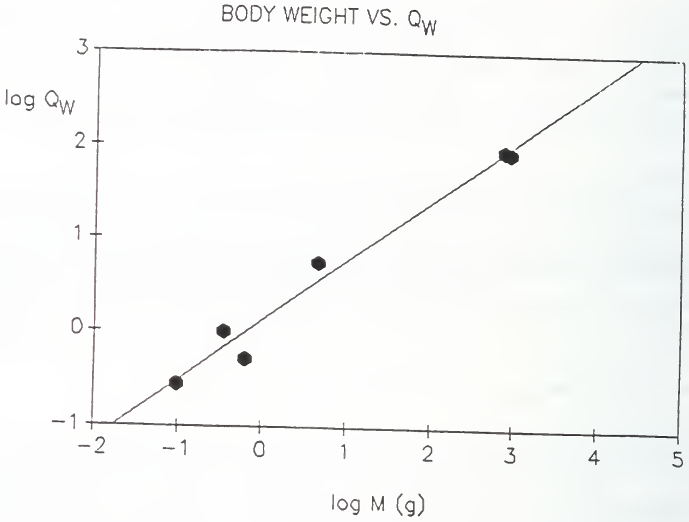


Figure 4.5

The logarithm of the water phase transport parameter in fish Q_W (L/d) as a function of logarithm of the body weight M (grams) of the fish.

The water phase transport parameter Q_W consists of a gill ventilation rate G_V (i.e. the volume of water passing through the gill compartment per unit of time) and a series of water phase transport processes in the fish. At this stage, these internal water phase transport parameters cannot be easily identified. They are therefore grouped together in a total internal water phase transport parameter Q_I. Since the gill ventilation rate and the internal water phase transport parameters apply in series it follows that

$$1/Q_W = 1/G_V + 1/Q_I \tag{4.50}$$

When the water phase transport parameter and the gill ventilation rate are known it is possible to determine the relative contribution of the gill ventilation rate to the overall transport parameter Q_W. For example, in a 780 g rainbow trout the gill ventilation (G_V) and the internal water phase transport processes (Q_I) contribute equally to the total water phase transport parameter (Gobas and Mackay, 1987). In smaller fish, the contribution of the gill ventilation to the total water transport parameter tends to be somewhat higher.

It can thus be concluded that the gill ventilation rate has an important influence on chemical uptake and elimination rates in fish. This is also reflected by the similarity in body-weight dependence of the total water phase transprt parameter and the gill ventilation rate. However, the gill ventilation rate does not fully control the bioconcentration kinetics, which was suggested by Norstrom et al. (1976) and Bruggeman et al. (1981) who proposed that k_1 equals $E_0.G_V/V_F$.

Norstrom et al. (1976) showed that the gill ventilation volumetric rate G_V is a function of the oxygen concentration in the water C_{OX} (ml of O_2/ml of water), the oxygen transfer efficiency across the gills E_{OX}, i.e. the fraction of oxygen delivered to the gill membrane which is actually absorbed by the fish, the metabolic rate coefficient A (ml of $O_2.h^{-1}.g^{-0.8}$) and the body weight of the organism M to the power 0.8, i.e.

$$G_V = A.M^{0.8}/(E_{OX}.C_{OX}) \tag{4.50A}$$

Combination of equations 4.42 to 4.44 and equations 4.48 and 4.49 provides a framework for the calculation of the uptake and elimination kinetics of (conservative) chemicals in various fish species. Only two parameters are required i.e. K_{OW} and the body weight of the organism M. It can be shown that especially for higher K_{OW} chemicals (i.e. log $K_{OW} > 3.5$) the effect of Q_L on the uptake and elimination kinetics is very small and can often be ignored with respect to Q_W. This further simplifies the estimation of the bioconcentration rate constants.

In section 7 we further explore these equations and demonstrate their use in the estimation of chemical uptake and elimination rate constants.

Oxygen Concentration in the Water

Norstrom et al. (1976) suggested a direct relationship between the ventilation volumetric rate (G_V) and the oxygen concentration in the water (C_{OX})i.e.

$$G_V = A.M^{0.8}/(E_{OX}.C_{OX}) \qquad (4.51)$$

where E_{OX} is the oxygen transfer efficiency across the gills, A is the metabolic rate coefficient (ml of $O_2.h^{-1}.g^{-0.8}$) and M is the body weight of the organism. Equation 4.51 demonstrates that a drop in the oxygen concentration in the water leads to higher gill ventilation rate. This higher ventilation rate allows the fish to extract a larger amount of water to meet its oxygen requirements. Earlier we have seen that the gill ventilation rate is an important regulating factor in chemical uptake and elimination kinetics. It thus follows that C_{OX} will also affect the rate constants for chemical uptake and elimination. The extent to which C_{OX} affects k_1 and k_2 depends on the relative importance of the gill ventilation rate and other transport processes on k_1 and k_2. For example, for hexachlorobenzene in a 750 g rainbow trout, the water phase transport parameter Q_W is made up of two processes i.e. the gill ventilation rate and other water transport processes, which are designated Q_I. Both processes contribute equally to Q_W. In other words, approximately half of the total water phase transport resistance $1/Q_W$ is due to the gill ventilation resistance i.e. $1/G_V$ and the other half is due to other water phase transport resistances i.e. $1/Q_I$. The lipid phase transport resistance $1/Q_L$ can be ignored with respect to K_{OW}/Q_W such that $1/k_2$ is approximately $V_L.K_{OW}/Q_W$ or $V_L.K_{OW}.(1/G_V + 1/Q_I)$. It thus follows that a drop in the C_{OX} by a factor of 2, which results in an increase in G_V by a factor of 2, leads to a reduction in $1/Q_W$ of 25% and an increase in Q_W and k_2 of 33%. This demonstrates that a variation in the gill ventilation rate or oxygen concentration does not necessarily result in a similar variation in k_2 and k_1. Only if Q_I is small compared to G_V and Q_L can be ignored an increase in gill ventilation rate is accompanied by a similar increase in k_2 and k_1. Unfortunately, only little information is available regarding the relative roles of Q_I, Q_L and G_V in various fish species and other aquatic organisms. It is therefore difficult to predict the

Table 4.3

Experimental (exp) and calculated (cal) kinetic parameters for 18 hydrophobic organic chemicals in rainbow trout.

Compound	log K_{OW}	E_{exp}	E_{cal}	$k_{1.CAL}$	k_2(exp)	k_2 (cal)
Ethylacetate	0.73	0.07	0.03	7	--	13
n-butanol	0.85	0.08	0.04	9	--	12
nitrobenzene	1.85	0.26	0.25	56	--	7.2
p-cresol	2.00	0.24	0.29	66	--	6.1
monochlorobenzene	2.84	0.56	0.48	109	--	1.4
2,4-dichlorophenol	2.93	0.52	0.49	111	--	1.2
decanol	3.09	0.59	0.51	134	--	0.85
pentachlorophenol	3.32	0.52	0.52	118	--	0.51
2,4,5-tetrachlorophenol	3.70	0.50	0.54	120	--	0.22
dodecanol	5.10	0.55	0.54	122	--	0.0089
2,5,2',5'-tetrachlorobiphenyl	6.00	0.5	0.54	122	--	0.0011
hexachlorobenzene	6.18	0.60	0.54	122	--	0.00074
fenvalerate	7.2	0.30	0.54	122	--	0.000071
mirex	7.5	0.20	0.54	122	--	0.000036
2,2' dichlorobiphenyl	4.9	--	0.54	122	0.014	0.014
2.3 dichlorobiphenyl	5.0	--	0.54	122	0.0079	0.011
2.5 dichlorobiphenyl	5.1	--	0.54	122	0.0047	0.0089
2,3,2',3' tetrachlorobiphenyl	5.6	--	0.54	122	0.0030	0.0028

effect on uptake and elimination kinetics as a result of varying oxygen levels in the water. As a first approximation it can be assumed that Q_1 and G_V are approximately equal and that Q_L can be ignored for relatively hydrophobic chemicals (log $K_{ow} > 4$). Finally, it should be noted that although oxygen levels may affect the bioconcentration kinetics, they do not affect the bioconcentration factor, i.e. the ratio of k_1 and k_2.

Temperature

The effect of temperature on chemical uptake kinetics in fish was studied by Neely (1979). This study demonstrated that in Yellow perch (Perca flavescens) the uptake rate constant k_1 increased by a factor of 4 when the temperature was raised from 5°C to 25°C. At the same time as the temperature increased the oxygen concentration in the water fell and the fish's metabolic rate coefficient (A) increased. It is therefore difficult to distinguish between the effects of temperature, oxygen concentration and the metabolic activity of the organisms. All of these factors are probably interrelated and play an important role in controlling the chemical uptake and elimination in aquatic organisms. Further studies are required to enable a more detailed analysis of the role of temperature.

5. BIOMAGNIFICATION

5.1 Introduction

Bioconcentration (i.e. bioaccumulation from the water only) in aquatic organisms was discussed in the previous section. This section will focus on the biomagnification or dietary bioaccumulation of chemicals. Biomagnification will be defined as the process by which ingestion of contaminated food results in a concentration of a contaminant in the organism, which exceeds that in the food. Biomagnification is often linked to food-chain accumulation, which is the phenomenon that chemical concentrations in organisms increase with increasing trophic level. Food-chain accumulation therefore refers to chemical dynamics in an ecological system. The chemical is transferred from one organism to another by predator-prey interactions. In this document, biomagnification refers to the concentration of a chemical from a food source by a single organism. Biomagnification at different trophic levels of the food-chain thus results in food-chain accumulation.

5.2 Biomagnification: Mechanism and Models

Bruggeman et al. (1981) introduced a kinetic model for dietary accumulation of contaminants in fish. This has been applied in various forms by other authors. This model views biomagnification as a balance of chemical uptake from ingested food and chemical elimination to the water via the gills. This model does not treat any other chemical excretion or egestion mechanisms. A schematic diagram of this model illustrating chemical transfer between the water, food and fish is presented in Figure 5.1. Chemical uptake from food is characterized by the feeding rate of the organism and the chemical's absorption efficiency from the ingested food. The feeding rate F expresses the amount of food consumed by the organism relative to the organism's body weight in a certain amount of time i.e. mass of food consumed/mass of the organism/time. The absorption efficiency E_f is the fraction of the ingested chemical that is actually absorbed by the organism from the food

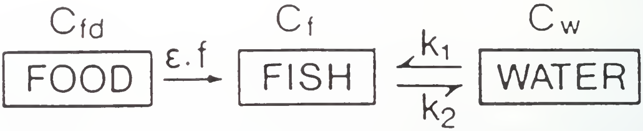


Figure 5.1

Schematic diagram of a model for dietary bioaccumulation in fish. This model was proposed by Bruggeman et al. (1981).

in the gastro-intestinal tract. The differential equation describing chemical uptake from food in the organism is

$$dC_F/dt = E_r.F.C_A - k_2.C_F \tag{5.1}$$

where C_A is the chemical concentration in the food, C_F is the concentration of the chemical in the organism and k_2 is the elimination rate constant to the water (via the gills). This equation can be integrated if the chemical concentration in the food is constant to give

$$C_F = (E_r.F/k_2).C_A.(1 - \exp(-k_2.t)) \tag{5.2}$$

Typically, biomagnification is measured in the laboratory. The experiments, which are described in more detail in section 8, involve exposing organisms to uncontaminated water and feeding them contaminated food at a constant feeding rate. Figure 5.2 illustrates the results of a typical biomagnification experiment showing the chemical concentration in the fish as a function of the exposure time.

At steady-state (i.e. when dC_F/dt equals zero or at infinite exposure time) the biomagnification factor K_M can be defined as

$$K_M = C_F/C_A = E_r.F/k_2 \tag{5.3}$$

Bruggeman et al. (1981 and 1984) measured biomagnification factors of some PCB congeners in goldfish and guppies and found values for K_M between 0 and 2. The biomagnification factor was further shown to increase with the 1-octanol-water partition coefficient for chemicals with a log K_{OW} up to 6. This increase was attributed to a decrease of the elimination rate constant k_2 with increasing K_{OW}.

A more detailed mechanism and model for chemical biomagnification was presented by Gobas et al. (1988). Similar to Bruggeman's food-uptake model, it treats the organism as a single homogeneous compartment, which is in continuous exchange with the water and its food. But in contrast to Bruggeman's model, it views biomagnification as a balance between the magnification (or concentration) of chemical in the food due to digestion of the food source and chemical depuration by chemical elimination to the water, chemical elimination into the faeces and metabolic transformation of the chemical.

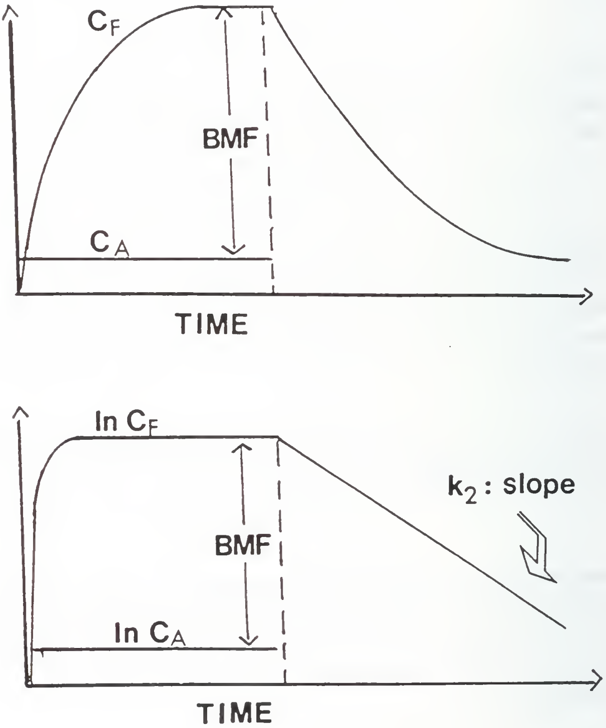


Figure 5.2

Illustrative example demonstrating that (i) - TOP - when a fish is exposed to a chemical concentration in the food (C_A), the chemical concentration in the fish (C_F) increases exponentially with time until it reaches a constant concentration (i.e. steady-state has been achieved); (ii) - BOTTOM - when a contaminated fish is exposed to clean, uncontaminated water and food (C_W = C_A = O) the chemical concentration in the fish decreases exponentially with time.

The expression describing simultaneous exchange of chemical between fish and water, and fish and food, and metabolic transformation, which is depicted in Figure 5.3, can be expressed as

$$dC_F/dt = k_1.C_W - k_2.C_F + k_A.C_A - k_E.C_F - k_R.C_F \qquad (5.4)$$

where C is concentration (mol/m^3), t is time (h), and the subscripts W refer to water, A to food, E to faeces, and F to the whole fish. The mass balance "envelope" is regarded as being the fish, but excluding the contents of the gill compartment and the gastro-intestinal (GI) tract. k_1, k_2, k_A and k_E are respectively the rate constants (h^{-1}) of chemical uptake from the water, elimination to the water, uptake from food, and elimination by egestion in the faeces, respectively, and k_R is the rate constant (h^{-1}) for metabolic transformation of the chemical in the fish.

Following the fugacity approach, equation 5.4 can also be written as

$$V_F.Z_F.df_F/dt = D_F.(f_W - f_F) + D_A.f_A - D_E.f_F - D_R.f_F \qquad (5.5)$$

where V is volume (m^3), Z is the chemical's fugacity capacity (mol/m^3.Pa) in a phase, and f is the chemical's fugacity (Pa). D_F is the overall transport parameter (mol/Pa.h) for chemical transfer between water and fish across the gills. D_A is the transport parameter for chemical uptake from food into the fish across the gastro-intestinal (GI)-tract. The transport parameter D_E (mol/Pa.h) describes chemical elimination in the faeces. D_R (mol/Pa.h) is the transformation parameter for metabolic transformation of chemical in the fish. The transport parameters D_F, D_A, and D_E include all transport processes involved in solute transfer between the water, food, and faeces, respectively, and the solute's final storage site in the fish.

Integration of equation 5.4 with a constant C_W and C_A, an initial C_F of zero and assuming a constant fish volume with time gives

$$C_F = (C_W.k_1/k_T + C_A.k_A/k_T)\{1 - \exp(-k_T t)\} \qquad (5.6)$$

where $k_T = k_2 + k_E + k_R$

Since C_F is f_F/Z_F, C_W is f_W/Z_W and C_A is f_A/Z_A, it can be shown that equation 5.6 is equivalent to the integrated form of equation 5.5 with a constant f_A and f_W and an initial f_F of zero, i.e.,

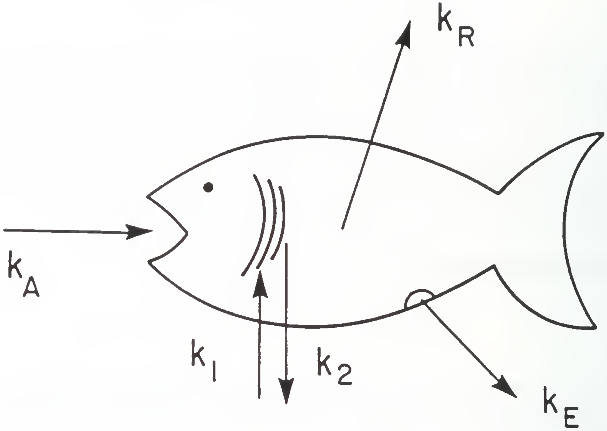


Figure 5.3

Schematic diagram of chemical uptake in fish from water (k_1) and food (k_A) and depuration by elimination to the water (k_2), elimination into the faeces (k_E), and metabolic transformation (k_R). See text leading to equation 5.4 for justification and definitions of terms and the mass balance envelope.

$$(f_W.D_F/D_T + f_A.D_A/D_T)(1 - \exp(- D_T.t/V_F.Z_V)) \qquad (5.7)$$

where $D_T = D_F + D_E + D_R$

and $k_1 = D_F/V_F.Z_W$ (5.8)

$k_2 = D_F/V_F.Z_F$ (5.9)

$k_A = D_A/V_F.Z_A$ (5.10)

$k_E = D_E/V_F.Z_F$ (5.11)

$k_R = D_R/V_F.Z_F$ (5.12)

Equations 5.6 and 5.7 show that at infinite exposure time a fish-water bioaccumulation factor, K_B can be defined for fish simultaneously exposed to contaminated water and food as

$$K_B = C_F/C_W = (Z_F/Z_W).\{[D_F/(D_F+D_E+D_R)] + [(f_A/f_W).(D_A/(D_F+D_E+D_R)]\} \qquad (5.13)$$

or

$$K_B = C_F/C_W = \{k_1/(k_2+k_E+k_R)\} + (C_A/C_W).\{k_A/(k_2+k_E+k_R)\} \qquad (5.14)$$

It also follows from equations 5.6 and 5.7 that in food uptake experiments, when fish are exposed to contaminated food but uncontaminated water, the ratio of fish to food concentrations, i.e., C_F/C_A or K_M, which is the biomagnification factor, can be expressed as

$$K_M = C_F/C_A = (Z_F/Z_A).\{D_A/(D_F+D_E+D_R)\} = k_A/(k_2+k_E+k_R) \qquad (5.15)$$

The bioconcentration factor, K_C, which is defined as the ratio of fish and water concentrations at infinite exposure time for fish exposed to contaminated water only (i.e., $C_A = f_A = 0$), is

$$K_C = C_F/C_W = (Z_F/Z_W).\{D_F/(D_F+D_E+D_R)\} = k_1/(k_2+k_E+k_R) \qquad (5.16)$$

Equations 5.13 to 5.16 demonstrate that K_B, K_M, and K_C are not solely determined by the thermodynamic quantities Z_F, Z_W and Z_A, which reflect the affinities of the chemical for the fish, water, and food, but also by the relative rates of chemical uptake from water and food, release to the water, egestion with the faeces, and metabolic transformation. Equation 5.14 shows that even when no metabolic transformation occurs ($D_R = 0$), the bioconcentration factor only reflects fish-water partitioning when D_E is small compared to D_F.

It thus follows, that in order to make reliable predictions about the bioaccumulation potential of hydrophobic chemicals in fish and the rate at which bioaccumulation is achieved in fish, knowledge is required about the processes controlling the exchange of solute between fish, water, food, and faeces.

II. Chemical Uptake from Food

Figure 5.4 illustrates that chemical uptake in fish from food can be viewed as the combined result of chemical transport through the GI-tract and the transfer of chemical between the GI-tract and the fish. Chemical transfer between the GI-tract and the fish can be described by

$$V_F.Z_F.df_F/dt = D_G.(f_G - f_F) \tag{5.17}$$

where D_G is the transport parameter for chemical transfer between the GI-tract and the chemical's final storage site in the fish and f_G is the chemical's fugacity in the GI-tract. We view this transfer process as being passive with a flux $D_G.f_G$ from the GI-tract to the fish and a reverse flux $D_G.f_F$ from the fish to the GI-tract. This is consistent with the concept that chemical transport is controlled by a concentration or fugacity gradient. If the fugacity in the fish exceeds the fugacity in the GI-tract, then the chemical tends to move from the fish to the GI-tract. This implies that the fish can eliminate chemicals by eating uncontaminated food. This reverse flux may also include a contribution from bile excretion. Bile excretion is expected to enhance chemical elimination into the faeces, thus causing the transport parameter D for fish-to-GI-tract transfer to exceed the transport parameter for GI-tract-to-fish transfer. However, for the present purpose we will assume that one transport parameter i.e. D_G describes chemical exchange between the fish and the GI-tract.

As has been discussed by Amidon et al. (1980,1983), chemical uptake from the GI-tract can often be satisfactorily described when the gut is treated as a well mixed reactor. The concentration or fugacity (f_G) in the GI-tract then serves to promote passive diffusion across the GI-tract to the blood and organs of the fish. The same fugacity applies to the egested faeces. A steady state mass balance on the GI-tract, including food intake and excretion, results in

$$D_G.(f_G - f_F) = D_I.f_A - D_O.f_G \tag{5.18a}$$

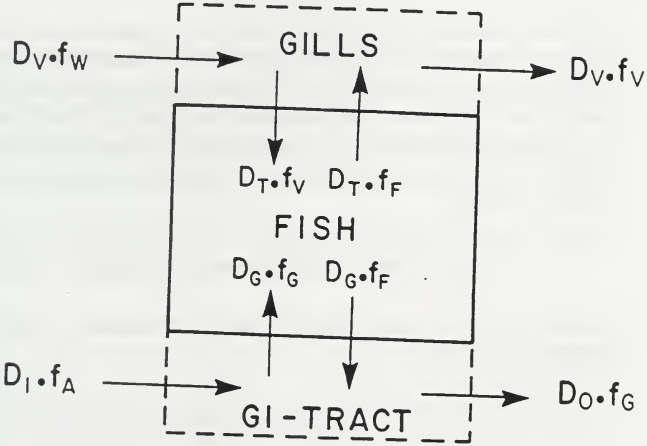


Figure 5.4

Schematic diagram of the chemical fluxes in and between the gill compartment, GI-tract, and the fish.

It thus follows that the GI-tract adopts a fugacity f_G of

$$f_G = (D_I.f_A + D_G.f_F)/(D_G + D_O) \tag{5.18b}$$

where $D_I.f_A$ is the flux with which chemical enters the GI-tract as a result of food consumption and $D_O.f_G$ is the flux with which chemical leaves the GI-tract as a result of faecal egestion. D_I is the transport parameter for chemical transport into the GI-tract and is the product of the feeding rate G_I (m^3/h) and the chemical's fugacity capacity in the food, Z_A, i.e., $G_I.Z_A$ such that $D_I.f_A$ equals $G_I.C_A$. The transport parameter for chemical transport out of the GI-tract, D_O is $G_O.Z_G$, where G_O is the faecal egestion rate (m^3/h), such that $D_O.f_G$ equals $G_O.C_G$. Substitution of equation 5.18b in equation 5.17 leads to

$$V_F.Z_F.df_F/dt = \{D_I.D_G/(D_O + D_G)\}.f_A - \{D_O.D_G/(D_O + D_G)\}.f_F \tag{5.19}$$

From equations 5.4, 5.5 and 5.19, it follows that the rate constants, k_A and k_E, are related to the transport parameters as

$$k_A = D_A/(V_F.Z_A) = \{D_I.D_G/(D_O + D_G)\}/(V_F.Z_A) \tag{5.20}$$
$$k_E = D_E/(V_F.Z_F) = \{D_O.D_G/(D_O + D_G)\}/(V_F.Z_F) \tag{5.21}$$

The absorption efficiency E_f is the ratio of the flux of chemical from the GI-tract into the fish, i.e. $D_G.(f_G - f_F)$, to the flux of chemical into the GI-tract, i.e. $D_I.f_A$ such that

$$E_f = D_G.(f_G - f_F)/D_I.f_A \tag{5.22}$$

Substitution of equation 5.18b into equation 5.22 shows that at the beginning of the exposure period, when no chemical is present in the fish and f_F is zero, the initial uptake efficiency, E_{f0}, is maximal, i.e.

$$E_{f0} = D_G/(D_O + D_G) \tag{5.23}$$

Alternatively, the absorption efficiency can be defined in terms of the ratio of the flux out of the GI-tract ($D_O.f_G$ or $G_O.C_G$) and into the GI-tract ($D_I.f_A$ or $G_I.C_A$) as

$$E_{f0} = 1 - (D_O.f_G/D_I.f_A) = 1 - (G_O.C_G/G_I.C_A) \tag{5.24}$$

which is equivalent to equation 5.22.

To convert from D-values to kinetic rate constants it is necessary to estimate Z_F and Z_A and also Z_G. This can be achieved by using the lipid or organic carbon content of the fish, food, and the faeces. Z_F, Z_A, and Z_G can then be equated to respectively, $L_F.Z_L$, $L_A.Z_L$, and $L_G.Z_L$ or $L_F.Z_O$, $L_A.Z_O$, and $L_G.Z_O$ (if $Z_L = Z_O$) where L_F, L_A, and L_G are the volume fractions of fish, food, and faeces with an affinity for the chemical equal to that of 1-octanol and Z_O is the chemical's fugacity capacity in 1-octanol.

A schematic diagram of the significance and relationships between the various D values and how these values control the absorption and excretion processes is presented in Figure 5.5.

The input of chemical associated with food is $D_I.f_A$. This mixes with the contents of the GI-tract and adopts a fugacity f_G. There are two removal processes from the GI-tract, i.e., absorption and egestion, characterized by D_G and D_O, respectively. The fraction of chemical absorbed is $D_I.f_A.D_G/(D_G + D_O)$ and that egested is $D_I.f_A.D_O/(D_G + D_O)$, which total $D_I.f_A$. Chemical also enters the GI-tract by diffusion from the fish at a rate $D_G.f_F$. This flux splits into $D_G.f_F.D_G/(D_G + D_O)$, which is reabsorbed, and $D_G.f_F.D_O/(D_G + D_O)$, which is eliminated by egestion. The total flux of chemical absorbed by the fish from food ingestion and reabsorption is therefore

$$D_G.f_G = (D_G.f_F + D_I.f_A).D_G/(D_O + D_G) \tag{5.25}$$

and the total flux of chemical eliminated by the fish in the GI-tract is

$$D_G.f_F = (D_G.f_F + D_O.f_F).D_G/(D_O + D_G) \tag{5.26}$$

The net flux into the fish is $\{(D_G.f_G)-(D_G.f_F)\}$ or $\{(D_I.f_A)-(D_O.f_F)\}.D_G/(D_O+D_G)$, which is equation 5.19, or $\{(D_A.f_A)-(D_E.f_F)\}$, which are the corresponding terms in equation 5.5. D_A and D_E thus represent the combined result of chemical flow in the GI-tract and chemical diffusion across the GI-wall.

A similar process occurs in the gills, where the gill ventilation rate is D_V, i.e., the product of the volumetric gill flow, G_V (m^3/h) and the chemical's fugacity capacity in water, Z_W. The rate of absorption is characterized by the transport parameter D_T. The gill water adopts a fugacity f_V, which can be deduced from a steady state mass balance similar to equations 17a and 17b namely

$$D_T.(f_V - f_F) = D_V.f_W - D_V.f_V \tag{5.27a}$$

or

$$f_V = (D_V.f_W + D_T.f_F)/(D_V + D_T) \tag{5.27b}$$

Figure 5.5 further illustrates that part of the chemical inflow $D_V.f_W$ is absorbed, whereas the other part is "by passed" in the gills and not absorbed. It follows that D_F is $D_V.D_T/(D_V + D_T)$. The significant difference between the absorption via the gills and from the GI-tract is that, whereas in the gills the inflow and outflow of chemical are characterized by a common D_V, in the GI-tract the inflow and outflow have different D values, D_I and D_O as a result of food mass loss and composition change.

Transfer Across the GI-Tract

To describe solute transfer between the GI-tract and the solute's storage sites in the fish the authors applied the approach originally suggested by Flynn and Yalkowsky (1972) and applied by several authors. GI-transfer is treated as a process in which the solute passes through a series of resistances in aqueous and lipid phases. Therefore, all D values applying to solute transport between the fish and the GI-tract in aqueous phases of the fish are grouped together in one term, D_W. Similarly solute transport in the lipid phases of the fish is expressed by a transport parameter, D_L. Each D term can be viewed as a product of a transport parameter, Q, with units of m^3/h, which combines all diffusion transfer terms, or flow rates (m^3/h) applicable to solute transfer in that phase and the fugacity capacity Z, i.e. $D_W = Q_W.Z_W$ and $D_L = Q_L.Z_L$. D_W and D_L can also be viewed as the conductivities of the water and lipid phase for chemical transport. The reciprocals $1/D_W$ and $1/D_L$ are the resistances of the water and lipid phase to chemical transport. Assuming that water and lipid transport processes apply in series and that transport resistances across lipid-water interfaces are negligible, the net transport of solute between the fish and the GI-tract can be expressed in terms of D_W and D_L as

$$1/D_G = 1/D_W + 1/D_L = 1/Q_W.Z_W + 1/Q_L.Z_L \tag{5.28}$$

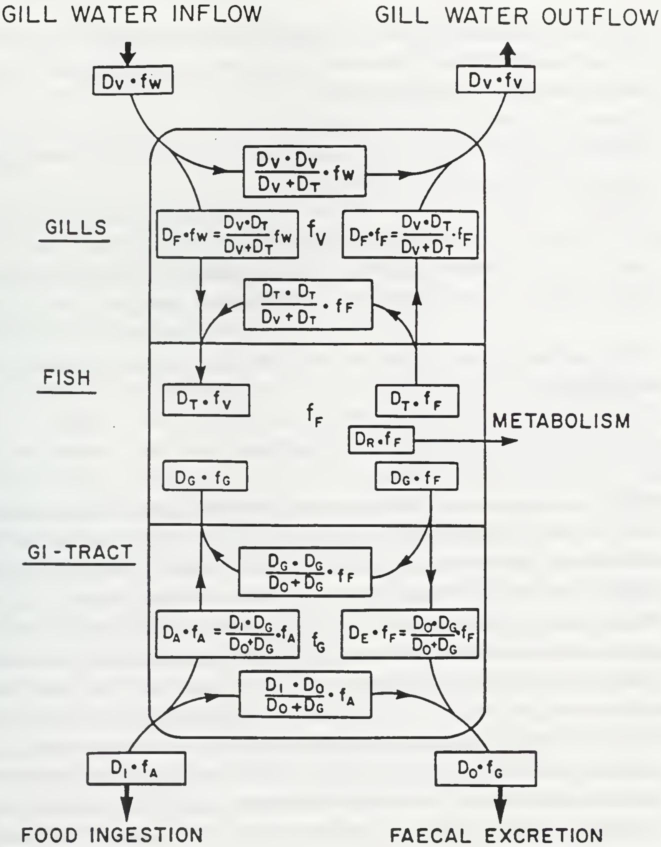


Figure 5.5

Detailed schematic diagram of the chemical fluxes in and between the gill compartment, the GI-tract, and the fish.

The transport parameters Q_W and Q_L in equation 5.25 for food uptake are specific to transfer of chemical across the GI-tract. They are not equal to the water and lipid phase transport parameters for fish-water exchange through the gills.

Substituting equation 5.25 for D_G, $G_I.L_A.Z_O$ for D_I, $G_O.L_G.Z_O$ for D_O, and K_{OW} for Z_O/Z_W in equations 5.20, 5.21 and 5.23, it follows that

$$1/k_A = (V_F/G_I).\{(G_O.L_G/Q_W).K_{OW} + (G_O.L_G/Q_L) + 1\} \tag{5.29}$$

$$1/k_E = (V_F.L_F/G_O.L_G).\{(G_O.L_G/Q_W).K_{OW} + (G_O.L_G/Q_L) + 1\} \tag{5.30}$$

$$1/E_{f0} = (G_O.L_G/Q_W).K_{OW} + G_O.L_G/Q_L + 1 \tag{5.31}$$

From equations 5.29 and 5.31, it follows that k_A and E_{f0} are related, as proposed by Bruggeman et al. (1981), as

$$k_A = E_{f0}.G_I./V_F \tag{5.32}$$

The reciprocal of k_A, i.e. $1/k_A$, can be viewed as the time needed to transport chemical from the food into the fish or as the total resistance for chemical transfer from the food into the fish. Likewise, $1/k_E$ is the time required to eliminate chemical from the fish into the faeces or the total resistance for chemical elimination to the faeces. The ratios $(V_F/G_I).(G_O.L_G/Q_W).K_{OW}$ and $(V_F/G_I).(G_O.L_G/Q_L)$ can be viewed as the solute's relative transport times in the water and lipid phases, respectively, of the fish or as the relative resistances that the solute encounters in the water and lipid phases of the fish on its route from the food phase in the GI-tract to the final storage site in the body lipid of the fish. When the solute's K_{OW} increases, and aqueous solubility thus decreases, the water phase of the fish can accommodate only a lower concentration of solute molecules. As a result, the time required to transport a certain amount of solute with this lower concentration increases. The resistance of the fish's water phase toward mass transfer thus increases, whereas it remains approximately constant in the lipid phase. For high K_{OW} chemicals, this implies that the uptake rate from food and elimination rate to the faeces and thus k_A, E_{f0} and k_E decrease with increasing K_{OW}. For low K_{OW} chemicals, uptake from food and elimination by excretion to the faeces is predominantly controlled by transport in lipid phases, and k_A, E_{f0}, and k_E are, therefore, expected to be approximately constant with respect to K_{OW}. From equation 5.31 it follows that the uptake efficiency E_{f0} follows a simple relationship with the 1-octanol-water partition coefficient i.e.

$$1/E_{f0} = A.K_{OW} + B \qquad (5.33)$$

where A is $G_O.L_G/Q_W$ and B is $(G_O.L_G/Q_L + 1)$. As will be discussed in more detail in section 5.3 Gobas et al. (1988) showed that experimental data fit this simple relationship with values for A and B of respectively $5.3.10^{-8}$ and 2.3.

Equation 5.33 demonstrates that by experimental measurement of E_{f0} for a series of chemicals of varying K_{OW} under controlled conditions, i.e. a constant feeding rate and no uptake of chemical from the water, it is possible to determine the fundamental kinetic parameters Q_W and Q_L. Knowledge of these parameters is invaluable for reliable estimation of organic chemical bioaccumulation from contaminated food.

One particular aspect of this food-uptake model deserves special attention since it has the ability to explain the phenomenon of biomagnification and food-chain accumulation observed for high K_{OW} chlorinated hydrocarbons in the field. As a result of digestion and absorption of food by the organism, the volume or volumetric flow rate of food continuously decreases while food is being transported through the GI-tract. This results in a faecal excretion rate G_O which is lower than the organism's food consumption rate, G_I. For example, for the guppy, the G_I/G_O ratio is approximately 3. This process causes the chemical's fugacity and concentration in the GI-contents (i.e. f_G or C_G) to increase, resulting in a fugacity in the GI-tract which exceeds the chemical's fugacity in the food (i.e. f_A). A further increase in the chemical's fugacity in the GI-tract may occur as a result of digestion of the ingested food in the GI-tract (e.g. hydrolysis of lipids), which may lower the affinity of the GI-contents for the chemical and thus reduce the chemical's fugacity capacity Z_G in the GI-tract. When the chemical's fugacities in food and fish are equal (i.e., fish and food are in chemical equilibrium), the chemical fugacity in the GI-tract will tend to exceed the fugacity in the food such that f_G/f_A equals D_I/D_O or $G_I.Z_A/G_O.Z_G$ (equation 5.18a), resulting in a net flux of chemical from the GI-tract into the fish. If the rate of chemical elimination to the water is small, and especially when smaller than the chemical elimination rate to the faeces ($D_W < D_E$), and, in addition, metabolic transformation is insignificant ($D_R < D_E$), the chemical's fugacity in the fish will tend to approach the fugacity in the GI-tract such that f_F/f_A equals D_I/D_O or $G_I.Z_A/G_O.Z_G$. Halogenated hydrocarbons with a high K_{OW} values (e.g. larger than 10^6) are an example of a class of chemicals which are extremely slowly metabolized and eliminated by fish. When a fish is exposed to food and water contaminated with this type of chemical and chemical fugacities in water and food are equal ($f_A = f_W$), which seems to be a reasonable assumption for fish feeding on organisms of the lower trophic levels in the environment, the chemical fugacity in the fish will tend to exceed the chemical's fugacity in the water (i.e. $f_F/f_W > 1$). As a result, the chemical's BAF (K_B) will exceed the BCF (K_C) and the fish-water partition coefficient, Z_F/Z_W, which is usually predicted from the chemical's K_{OW} as $L_F.K_{OW}$. The extent to which K_B exceeds K_C or f_F/f_W over 1 depends on the relative values of D_A and D_E or G_I and G_O and the lipid content of the food. Bioaccumulation factors of high K_{OW} chemicals in Lake Ontario fish have indeed been shown to exceed fish-water partition coefficients (Oliver and Niimi 1988) and calculated fugacities of these chemicals in fish to be higher than fugacities in the water (Connolly and Pedersen 1988). As demonstrated by Connolly and Pedersen (1988) and Clark et al. (1988), this increase in fugacity may take place at every trophic level of the food-chain, where one organism feeds on another organism of a "lower" trophic level and can thus be viewed as the driving force of what is generally referred to as food-chain accumulation.

5.3 The Kinetics of Chemical Uptake from Food Consumption

The rate at which organisms absorb chemicals by food consumption is generally considered to be a function of the amount of food ingested per unit of time, i.e. the feeding rate of the organism (G_I), the concentration of chemical in the food (C_A) and the extent to which the chemical is extracted from the food, i.e. the absorption efficiency E_{f0}. It can be expressed as $k_A.C_A$ where k_A is given by

$$k_A = E_{f0}.G_I/V_F \tag{5.34}$$

The feeding rate G_I is a property of the organism and the abundance of food in its environment. In the field, it is therefore controlled by a variety of factors; but in the laboratory it can often be controlled. For many fish species, the daily food consumption equals approximately 1 to 2 % of the fish's body weight. As a result, the ratio of G_I/V_F can often be estimated as 0.01 to 0.02 d^{-1}. Since G_I/V_F is a property of the fish and therefore independent of type of contaminants in the food, it is believed to be the same for all chemicals.

The chemical dependent variable in equation 5.34 is the absorption efficiency or assimilation efficiency E_{f0}. Various authors have investigated relationships between E_{f0} and chemical properties such as K_{OW}, molecular volume and molecular weight.

Niimi and Oliver (1988) showed that chemicals with a molecular weight greater than 600 or a molecular volume larger than 0.3 nm^3 have very low absorption efficiencies and are thus poorly absorbed by fish. However, it was also concluded that neither the molecular weight nor the molecular volume could provide a reliable relationship for the estimation of absorption efficiencies in fish. Opperhuizen and Schrap (1988) also did not find a relationship between the dietary uptake efficiency and physicochemical parameters of the chemical.

Muir and Yarechewski (1988) noticed an inverse relationship between the dietary absorption efficiency and the 1-octanol-water partition coefficient for chemicals with a log K_{OW} larger than 7. Although the spread in the experimental data did not allow a meaningful correlation between E_{f0} and K_{OW} it was concluded that the absorption efficiency E_{f0} falls with increasing K_{OW} for chemicals with very high K_{OW}. It was suggested that reduced membrane permeation, due to the larger molecular size, is the cause of the low absorption efficiencies of the higher K_{OW} chemicals.

Gobas et al. (1988) investigated the relationship between absorption efficiency (E_{f0}) and the 1-octanol-water partition coefficient. However, in contrast to the previously discussed studies, the analysis was based exclusively on experimental data from studies in which fish were exposed to contaminated food for a relatively long period of time with a constant feeding rate. Data from studies in which fish were exposed to a single oral dose were not taken into account. In addition, only data for single compounds were considered. Figure 5.6 illustrates the observed relationship between E_{f0} and log K_{OW}.

Although there is a considerable spread in the data, Figure 5.6 suggests that, for chemicals with a log K_{OW} up to approximately 7, the uptake efficiency from food is constant. For chemicals with a log K_{OW} exceeding 7, the uptake efficiency from food falls with increasing K_{OW}. The following empirical relationship between the uptake efficiency E_{f0} and K_{OW} was observed:

$$1/E_{f0} = 5.3.10^{-8}.K_{OW} + 2.3 \qquad (5.35)$$

This correlation was derived by fitting experimental data to equation 5.34, i.e.

$$1/E_{f0} = A.K_{OW} + B \qquad (5.36)$$

using non-linear regression to fit the constants A and B. This resulted in values of 5.3 (+/-1.5). 10^{-8} for A and of 2.3 (+/-0.3) for B where the confidence intervals have a 95% probability. In Figure 5.6, the solid line represents the correlated behaviour of E_0 with respect to log K_{OW} and the broken lines indicate the asymptotic 95% confidence intervals of the proposed correlations. Equation 5.36 is similar to equation 5.31, which followed from the food uptake model discussed earlier in this section. Equation 5.35 thus validates the food uptake model and also presents a practical correlation for the estimation of chemical uptake efficiencies in fish.

Gobas et al. (1988) also estimated the transport parameters Q_W and Q_L for chemical uptake from food in various fish species. The values are listed in Table 5.1.

It is believed that equation 5.35 is the most accurate relationship for the absorption efficiency of organic chemicals in fish. However, Figure 5.6 illustrates a considerable spread in the data. The transport parameters Q_W and Q_L may therefore also contain a relatively large error. The need for more accurate data on the chemical absorption efficiency in aquatic organisms is therefore stressed. Better experimental data will undoubtedly lead to more precise relationships between absorption efficiencies and physicochemical parameters. And thus, to more reliable predictive models for chemical uptake kinetics from food.

5.4 Relationship Between the Biomagnification Factor and the 1-Octanol-Water Partition Coefficient

Information regarding the relationship between the biomagnification factor and physicochemical parameters, such as the 1-octanol-water partition coefficient are rather scarce. This is probably the result of the experimental difficulties associated with the measurement of biomagnification factors. Biomagnification factors are usually small numbers (in the order of 2 to 4), representing the relatively small differences between chemical concentrations in the organism and in its food. Frequently, it is observed that the experimental error in the measurements of the individual concentrations in the organism and the food are too large to notice a statistical difference in concentration (Macek et al. 1979). As a result biomagnification factors are rarely reported. Two studies, however, are worthwhile mentioning. Bruggeman et al. (1984) measured biomagnification of some PCB congeners in guppies in laboratory experiments. In female guppies (Poecilia reticulata) it was observed that the biomagnification factor K_M increased with log K_{OW} from 0.03 for 2,5-dichlorobiphenyl (log K_{OW} is 5.2) to 1.2 for 2,2',4,4',5,5'-hexachlorobiphenyl (log K_{OW} is 7.0).

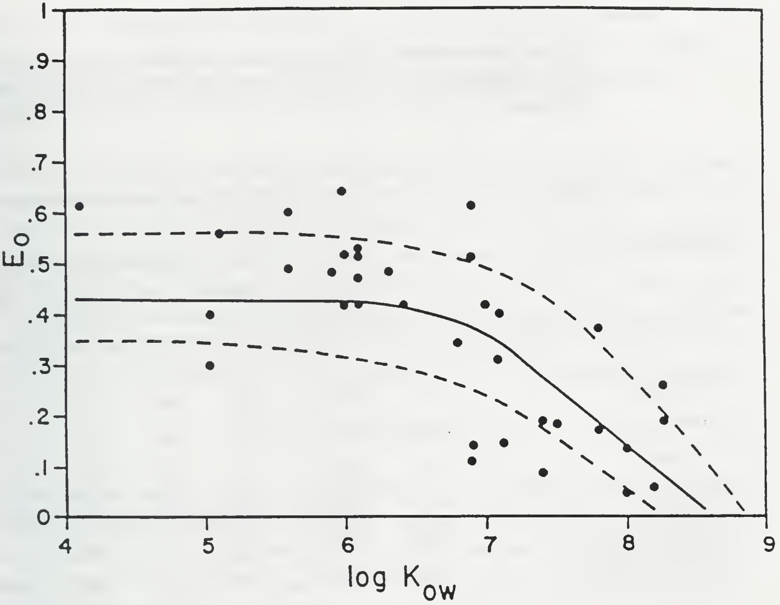


Figure 5.6

Observed absorption efficiencies (E_o) of hydrophobic organic chemicals in fish as a function of the 1-octanol-water partition coefficient (log K_{ow})

Table 5.1: Fish wet weight or volume (V_F), lipid content (L_F), reported feeding rate (g) and the volumetric feeding rate (G_I) and calculated water- and lipid phase transport parameters for gastro-intestinal absorption (resp. Q_W and Q_L) of hydrophobic organic chemicals in various fish species.

FISH SPECIES	V_F (g)	L_F	g (g/g/d)	G_I (μL/d)	Q_W (L/d)	Q_L (μL/d)
GUPPY male	0.1	0.022	0.02	9.2	1.4	0.058
GUPPY female	0.35	0.039	0.02	32	9.1	0.36
GOLDFISH	4.5	0.033	0.01	210	48	2.0
SALMON	3.4	0.046	0.039	610	198	8.0
RAINBOW TROUT	0.75	0.085	0.015	52	31	1.3
FATHEAD MINNOW	1.0	0.036	0.015	69	18	0.71

However, for PCB congeners of higher K_{ow} the biomagnification factor decreased to 0.7 for decachlorobiphenyl (log K_{ow} is 8.3). In male guppies a similar trend was observed but biomagnification factors increased from 0.14 for dichlorobiphenyl to approximately 6 for hexachlorobiphenyl.

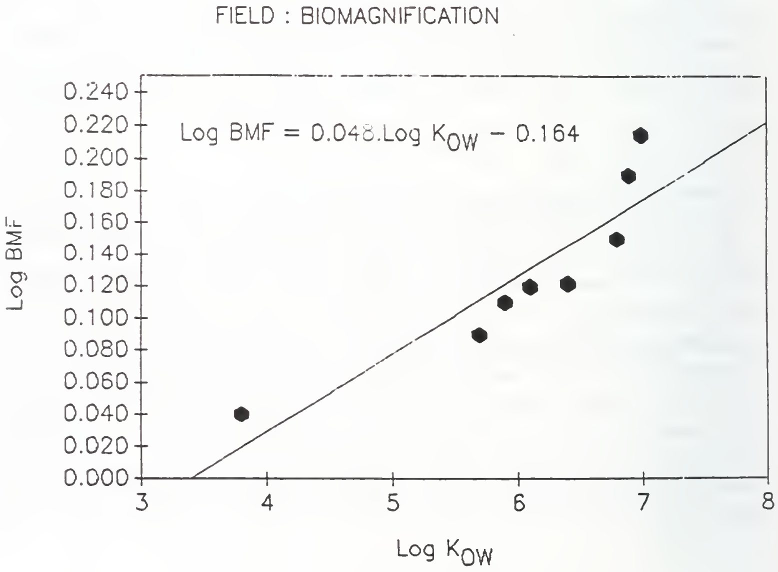
Gobas and Youssuf (1988, unpublished) recently analyzed reported field studies on food-chain accumulation in Lake Ontario (Oliver and Niimi 1980). The reported experimental data were used to derive ratios of chemical concentrations in predator and prey (i.e. food). These ratios can be considered biomagnification factors. The predator/prey concentration ratios were then classified according to the K_{ow} of the chemical and averaged to arrive at a single value for BMF for each K_{ow}. Each BMF was therefore the average of approximately 250 predator/prey concentration ratios. The results are illustrated in Figure 5.7 and demonstrate a linear correlation between log BMF and log K_{ow} for chemicals with a log K_{ow} between 3.8 and 7.5. The correlating equation is:

$$\log K_M = 0.048.\log K_{ow} - 0.164 \qquad (5.37)$$

It shows that the biomagnification of chemicals of chemicals with a log K_{ow} of 4.0 is approximately 1.07. In other words, biomagnification will result in a concentration in the organism which is at the most 7% higher than the concentration in the food. Clearly, biomagnification is not an important process. For a chemical with a log K_{ow} of 7.0 equation 5.37 indicates that the biomagnification factor is approximately 1.5. Now, biomagnification can result in a chemical concentration in the organisms that is 50% higher than that in the food. If this biomagnification factor applies at, for example, four levels of the food-chain, the concentration at the fourth trophic levels will be 1.5 x 1.5 x 1.5 x 1.5, i.e. approximately 5 times higher than in the original food source. Biomagnification for a chemical with a log K_{ow} of 7.0 is thus a more important process than it is for a chemical with a log K_{ow} of 4.0. It should be noted that equation 5.37 is an empirical equation. It should therefore only be used within the range of 1-octanol-water partition coefficients, for which it has been derived.

Figure 5.7

The logarithm of the biomagnification factor, log BMF (i.e. the ratio of chemical concentrations in predator over that in the prey) as a function of the logarithm of the 1-octanol-water partition coefficient (log K_{ow}).



6. FOOD CHAIN TRANSFER

6.1 Introduction

Food-chain transfer is the phenomenon in which the consumption of one organism by another organism results in the transfer of contaminants in the prey to the predator. When the contaminant tends to biomagnify in the organism, thus resulting in a chemical concentration in the organism exceeding that in the food, it is possible that predator-prey interactions will lead to an increase in chemical concentrations in the organism with increasing trophic level. This can be referred to as food-chain accumulation. Food-chain accumulation is thus the result of a series of biomagnification effects. Biomagnification refers to a single organism, and is chemical and organism specific but independent on predator-prey relationships. Food-chain accumulation, however, is the result of the chemical's ability to biomagnify in a series of organisms. It is therefore a function of (i) the chemical, (ii) the organisms in the food-chain and (iii) the interactions between the organisms in the food-chain. The terms biomagnification and food-chain accumulation are often treated as synonyms. This can be misleading. In order to prevent misunderstandings with respect to the terminology we will distinguish between the two phenomena and use the terms biomagnification and food-chain accumulation as defined above.

Interest in food-chain accumulation in aquatic systems arose as a result of the very high concentrations of PCBs and DDT observed in some fish species. Various authors have investigated food-chain transfer and accumulation in the laboratory and in the field. Laboratory experiments usually involved the exposure of a series of organisms of different "trophic" level to an aqueous solution of a chemical(s). In virtually all cases, the body burden in the organisms due to dietary exposure was statistically indistinguishable from that due to aqueous exposure, thus suggesting a lack of food-chain transfer (Macek et al. 1979, Kay 1984). Some notable exceptions were the very high K_{ow} chemicals such as DDT, PCB and mirex.

Field studies are more conclusive about the role of food-chain accumulation. Connolly and Pedersen (1988) showed that the fugacity of PCB and some pesticides in certain fish species exceeded the fugacity of the chemical in the water. Since bioconcentration i.e. chemical partitioning between the fish and the water would result in equal fugacities in water and fish, the authors explained the higher fugacities in the fish by biomagnification. Connolly and Pedersen further showed that the ratio of fish to water fugacities increased with increasing trophic level, thus demonstrating food-chain accumulation.

Oliver and Niimi (1988) reported a food-chain accumulation study in Lake Ontario. This study also indicates that higher K_{OW} chemicals such as PCB bioaccumulate more in organisms of higher trophic level. Bioaccumulation factors in the top predators, i.e. salmonids were also observed to exceed the bioconcentration factors thus suggesting chemical uptake from food to be an important factor controlling the body burden in these organisms.

6.2 Mechanisms and Models

We discuss here two food-chain models, which have been applied to predict food-chain accumulation. First is a concentration-based model while the second is fugacity-based.

The first model was originally developed by Thomann and coworkers (e.g. Thoman and Connolly 1984). It is also known as the Manhattan College Model or WASTOX (Connolly and Winfield 1986). This model assumes a steady-state of the chemical in the organisms (C_F), in the water (C_W) and the food of the organism or prey (C_A). This can be described by the following equation

$$C_F = (k_u/k_d).C_W + (E^{*}.F^{*}/k_d).C_A \tag{6.1}$$

where k_u is the uptake rate of chemical from the water, k_d is the overall loss rate (including growth and excretion), E^{*} is the assimilation efficiency of chemical in food and F^{*} is the rate of food consumption. Dividing equation 6.1 by C_W yields

$$N_{i+1} = (k_u/k_d) + (E^{*}.F^{*}/k_d).N_i \tag{6.2}$$

where N_{i+i} is the field bioaccumulation factor for the predator and N_i is the field bioaccumulation factor for the prey. The left hand term in equation 6.2 represents chemical uptake from the water, i.e. bioconcentration. The ratio (k_u/k_d) is thus the bioconcentration factor $N_{w,i+1}$ in the predator. The right hand term in equation 6.2 describes the contribution of food consumption to the bioaccumulation factor. It is often simplified to $q.N_i$ such that equation 6.2 can be rewritten as

$$N_{i+1} = N_{W,I+1} + q_{i+1,i}.Ni \qquad (6.3)$$

where

$$q_{i+1,i} = (E^{*}.F./k_d) \qquad (6.4)$$

The food-chain is viewed as consisting of four levels. Organisms of the first trophic level are considered to be in equilibrium with the water only. In that case chemical uptake from food consumption is considered insignificant, i.e. q_1 is zero and

$$N_1 = N_{w1} = (k_u/k_d) \qquad (6.5)$$

Organisms of the second trophic level are assumed to feed exclusively on organisms of the first trophic level. The bioaccumulation factor for organisms of the second trophic level is therefore

$$N_2 = N_{w2} + q_{2,1}.N_{w1} \qquad (6.6)$$

Organisms of the third trophic level consume organisms of the second trophic level, thus resulting in a bioaccumulation factor of

$$N_3 = N_{w3} + q_{3,2}.N_{w2} + q_{3,2}.q_{2,1}.N_{w1} \qquad (6.7)$$

Finally, organisms of the fourth trophic level are viewed to feed on organisms of the third trophic level such that

$$N_4 = N_{w4} + q_{4,3}.N_{w3} + q_{4,3}.q_{3,2}.N_{w2} + q_{4,3}.q_{3,2}.q_{2,1}.N_{w1} \qquad (6.8)$$

Equations 6.5 to 6.8 show the cumulative effect of transfer of chemical from the lower levels of the food-chain to the top predator level. The equations require information regarding the uptake and elimination rate constants, the assimilation efficiency and the rate of food consumption. The uptake rate constant k_u is believed to be a function of the respiration rate of the animal such that

$$k_u = E_w.R/(E_{ox}.C_{ox}) \qquad (6.9)$$

where R is the respiration rate, E_w and E_{ox} are the efficiencies for chemical uptake from water and oxygen uptake and C_{ox} is the oxygen concentration in the water. For chemicals of log K_{OW} between 3 and 6.2 it is assumed that E_w equals E_{ox}. C_{ox} is assumed to be 10 mg/L and R is believed to be function of the body weight M (grams) of the organism, i.e.

$$R = 10.M^{-0.2} \tag{6.10}$$

The rate constant k_d is estimated from the bioconcentration factor k_u/k_d. The bioconcentration factor is assumed to be a fraction, i.e. the lipid fraction of the organism L_F, of the 1-octanol-water partition coefficient K_{OW}, i.e. $L_F.K_{OW}$. The rate constant k_d is thus calculated as

$$k_d = k_u/(L_F.K_{OW}) \tag{6.11}$$

From a review of the literature the assimilation efficiency E is taken to be 0.6 for all chemicals and in all organisms. Further, organism specific values for the weight, lipid fraction, growth rate and consumptions rate are required to perform the model calculations. Typical values for q are

$$q_2 = 0.18/(0.04 + (2.10^5/K_{OW})) \tag{6.12}$$

$$q_3 = 0.024/(0.0046 + (9000/K_{OW})) \tag{6.13}$$

$$q_4 = 0.012/(0.0025 + (2500/K_{OW})) \tag{6.14}$$

which were reported by Connolly and Pedersen (1988). This model indicates that at log K_{OW} less than 4, the uptake of chemical from food is insignificant. At higher K_{OW} values, chemical uptake from food becomes more important. This is the result of a decreasing excretion rate, which reduces the depuration of chemical assimilated from food. At log K_{OW} larger than 7 the models predicts that fish growth controls the "loss rate" of the chemical, with no further increase in biomagnification factor. Thomann (1988) and Connolly and Pedersen (1988) have demonstrated that this model can be applied successfully.

The second model for food-chain accumulation applies the bioaccumulation model developed by Gobas et al. (1988) at every level of the food-chain. Since this bioaccumulation model has been discussed in detail in section 5 we will only show how these equations can be applied to describe food-chain accumulation. Similarly to the Thomann model it is assumed that the fish are at steady-state. Equation 5.6 demonstrates that the chemical concentration in the fish C_F can then be expressed as

$$C_F = C_W.[k_1/(k_2+k_E+k_R)] + C_A.[k_A/(k_2+k_E+k_R)] \tag{6.16}$$

and the bioaccumulation factor K_B is

$$K_B = C_F/C_W = \{k_1/(k_2+k_E+k_R)\} + (C_A/C_W).\{k_A/(k_2+k_E+k_R)\} \tag{6.17}$$

Growth of the fish can also be included in equations 6.16 and 6.17, for example in the form of a pseudo rate constant k_G which is $dV_F/(V_F.dt)$. This would change equation 6.17 to

$$K_B = C_F/C_W = \{k_1/(k_2+k_E+k_R+k_G)\} + (C_A/C_W).\{k_A/(k_2+k_E+k_R+k_G)\} \tag{6.18}$$

Growth, however, results not only in an increase in the size of an organism, but also changes the organism's position in the food-chain. Since it is difficult to distinguish between an increase in body weight and trophic position it is convenient to (initially) ignore the possible effects of growth. It is thus assumed that every organism in the food-chain has a constant body mass i.e. k_G is zero. The rate constants k_1 and k_2 can be derived from equations 4.19 and 4.18, i.e.

$$1/k_1 = V_F.\{1/Q_W + (1/Q_L.K_{OW})\} \tag{6.19}$$

$$1/k_2 = V_L.\{(K_{OW}/Q_W) + 1/Q_L\} \tag{6.20}$$

where V_F is the volume of the organism and V_L is the lipid volume of the organism and Q_W (L/d) and Q_L (L/d) can be estimated from the weight of the organism M (g):

$$Q_W = 1.4.M^{0.6} \tag{6.21}$$

$$Q_L = 0.014.M^{0.6} \tag{6.22}$$

The rate constants k_A and k_E can be derived from equations 5.29 and 5.30, i.e.

$$1/k_A = (V_F/G_I).(5.3.10^{-8}.K_{OW} + 2.3) \tag{6.23}$$

$$1/k_E = (V_F.L_F/G_O.L_G).(5.3.10^{-8}.K_{OW} + 2.3) \tag{6.24}$$

At this stage it is preferable to substitute the actual feeding rates G_I and faecal egestion rates G_O of the organisms in the food chain. If these values are not available, an average daily consumption rate of 1% of the organism's body weight is often a reasonable estimate. In that case V_F/G_I in equation 6.23 is 100 d. A reasonable estimate of the average "faecal egestion rate" for widely differing organisms in a food-chain is probably 25% of the feeding rate.

Equations 6.16 to 6.24 show that bioaccumulation and biomagnification at every level of a food-chain can be estimated from the K_{OW} of the chemical, the body weight M of the organism and the lipid content L_F or lipid volume V_L. This can be illustrated by applying these equations to a simplified generic food-chain model similar to that used by Thomann. This generic food-chain model consists of four trophic levels represented by the body weights of the organisms, i.e. 1st trophic level ($M < 0.1$g), 2nd trophic level ($M = 0.1$ g), 3rd trophic level ($M = 10$ g) and 4th trophic level ($M = 1000$ g). Similarly to the Thomann model it will be assumed that for organisms of the first trophic level chemical uptake from food and chemical depuration other than to the water are insignificant, i.e. k_A and k_E are zero. Also, k_R will be assumed to be zero. It thus follows that the concentration of a chemical in organisms of the first trophic level C_{F1} is

$$C_{F1} = L_{F1}.K_{OW}.C_W \tag{6.25}$$

If the lipid content of this 1st trophic level organism L_{F1} is 0.01 and C_W is 1 pg/L, C_{F1} will be 100 pg/kg for a chemical with a log K_{OW} of 4 and 100,000 pg/kg or 100 ng/kg for a chemical with a log K_{OW} of 7.

Organisms of the 2nd trophic level ($M = 0.1$ g) are exposed to both contaminated water and contaminated food by feeding on organisms of the 1st trophic level. To determine the concentration in the 2nd trophic level organism (C_{F2}) it is necessary to calculate the rate constants k_1, k_2, k_A and k_E. The metabolic transformation rate constant k_R will again be assumed to be zero. If L_{F2} is 0.02 it follows from equations 6.19 to 6.24 that for log K_{OW} of 4:

$k_1 = 3480$ d^{-1}

$k_2 = 17.4$ d^{-1}

$k_A = 0.00435$ d^{-1}

$k_E = 0.00109$ d^{-1}

C_{F2} can now be calculated by substituting these rate constants in equation 6.16 and by substituting C_{F1} for C_A:

$$C_{F2} = 1\text{pg/L}.(3480/(17.4+0.00109)) + 100\text{pg/Kg}.(0.00435/(17.4+0.00109)) = 200.024 \text{ pg/kg} \tag{6.26}$$

For log K_{ow} of 7 the rate constants are:

$k_1 = 3521 \ d^{-1}$
$k_2 = 0.0176 \ d^{-1}$
$k_A = 0.00353 \ d^{-1}$
$k_E = 0.000883 \ d^{-1}$

It thus follows that

$$C_{F2} = 1 \text{ pg/L}.(3521/(0.0176+0.000883)) + 100{,}000 \text{ pg/Kg}.(0.00353/(0.0176+0.000883)) = 210{,}000 \text{ pg/kg} \tag{6.27}$$

For organisms of the 3rd trophic level ($M = 10$ g and $L_F = 0.04$), which feed on organisms of the second trophic level the calculations for a chemical of log K_{ow} of 4 are:

$k_1 = 550 \ d^{-1}$
$k_2 = 1.38 \ d^{-1}$
$k_A = 0.00435 \ d^{-1}$
$k_E = 0.00109 \ d^{-1}$

$$C_{F3} = 1 \text{ pg/L}.(550/(1.38+0.00109)) + 200.024 \text{ pg/Kg}.(0.00435/(1.38+0.00109)) = 399 \text{ pg/kg} \tag{6.28}$$

For a chemical with a log K_{ow} of 7:

$k_1 = 557 \ d^{-1}$
$k_2 = 0.00139 \ d^{-1}$
$k_A = 0.00353 \ d^{-1}$
$k_E = 0.000883 \ d^{-1}$

$$C_{F3} = 1\ \text{pg/L}.(557/(0.00139+0.000883)) + 206{,}000\ \text{pg/Kg}.(0.00353/(0.00139+0.000883)) = 571{,}000\ \text{pg/Kg} \qquad (6.29)$$

Finally, for organisms of the 4th trophic level ($M = 1000$ g, $L_F = 0.10$), which feed exclusively on organisms of the 3rd trophic level, the model predicts the following for a chemical of log K_{OW} of 4:

$k_1 = 87.4\ d^{-1}$
$k_2 = 0.0883\ d^{-1}$
$k_A = 0.00435\ d^{-1}$
$k_E = 0.00109\ d^{-1}$

$$C_{F4} = 1\ \text{pg/L}.(87.4/(0.0883+0.00109)) + 399\ \text{pg/Kg}.(0.00435/(0.0883+0.00109)) = 998\ \text{pg/Kg} \qquad (6.30)$$

For a chemical with a log K_{OW} of 7 the model predicts:

$k_1 = 88.3\ d^{-1}$
$k_2 = 8.83.10^{-5}\ d^{-1}$
$k_A = 0.00353\ d^{-1}$
$k_E = 0.000883\ d^{-1}$

$$C_{F4} = 1\ \text{pg/L}.(88.3/(8.83.10^{-5}+0.000883)) + 500{,}000\ \text{pg/Kg}.(0.00353/(8.83.10^{-5}+0.000883) = 2{,}080{,}000\ \text{pg/Kg} = 2.08\ \text{ug/Kg} \qquad (6.31)$$

In summary, the model illustrates that in a four step food-chain a chemical of log K_{OW} of 4 will reach concentrations in the four trophic levels of respectively of 100, 200, 399 and 998 pg/Kg. It appears that food-chain accumulation occurs since the concentration in the organisms increases with every step in the food-chain. However, this is not necessarily true. The increase in concentration with trophic level may merely reflect the increase in the lipid content of the organisms from 0.01 (i.e. 1%) to 0.1 (i.e. 10%). This becomes apparent when the concentrations are expressed on a lipid weight basis, i.e. as the amount of chemical (in pg) per weight of lipid (in Kg). This can easily be achieved by dividing the chemical concentration by the respective lipid fraction L_F. When expressed on a lipid weight basis the concentrations at the four trophic levels are respectively 10,000, 10,000, 9975 and 9980 pg/Kg. It can thus be concluded that the chemical concentrations in the lipid tissue of the organisms at the four trophic levels are essentially the same. The organisms are therefore at an equal chemical fugacity and biomagnification does not occur.

For chemicals of log K_{ow} of 7 the situation is different. Expressing the chemical concentration at the four trophic levels on a lipid weight basis results in values of $1.0.10^7$, $1.05.10^7$, $1.43.10^7$ and $2.08.10^7$. Clearly, the chemical concentration increases with increasing trophic level. The calculations further show that the biomagnification factor is larger for larger size organisms. For the relatively small organism of the second trophic level the biomagnification factor is 1.05. But for the large size organisms of the fourth trophic level the biomagnification factor is 1.43. This corresponds with experimental findings in Lake Ontario (Oliver and Niimi, 1988). The reason for the low biomagnification factor in small organisms is that chemical exchange with the water is relatively more important than chemical uptake from food for small organisms than it is for larger organisms. With increasing size of the organism, chemical uptake from food tends to play a more important role and chemical exchange with the water a less important role. This results in a larger biomagnification effect in larger organisms.

It transpires that chemicals with a log K_{ow} below 5 do not tend to accumulate in the food-chain. These chemicals exchange rapidly with the water and the organism concentration will reflect the chemical concentration in the water. Organisms exposed to the same concentration in the water will therefore contain the same chemical concentration in their lipids.

Food-chain accumulation becomes important for chemicals with a log K_{ow} above 5. It is particularly important for chemicals with log K_{ow} of 6 to 8. This is mainly the result of the fact that chemical exchange between the water and the organism is extremely slow such that chemicals taken up from the food cannot be eliminated to the water. The organism does not reflect the water concentration. In addition, due to their extreme hydrophobicity the dissolved (available) chemical concentration in the water tends to be very low and chemical concentration in (lipid rich) food sources are relatively high. This makes chemical uptake from food and food-chain accumulation a more important process than chemical bioconcentration from the water.

7. METHODS FOR ASSESSING THE BIOCONCENTRATION, BIOMAGNIFICATION, BIOACCUMULATION AND FOOD-CHAIN TRANSFER OF ORGANIC CHEMICALS IN AQUATIC ORGANISMS

7.1 Introduction

This chapter gives step by step instructions for the calculation of bioconcentration, biomagnification and bioaccumulation factors and rate constants of organic chemicals in aquatic organisms. It also provides methods for estimating food-chain transfer. It is designed to assist the OME in the assessment of the exposure of organisms to virtually all organic chemicals, presently in use in Ontario. The calculated values derived from this manual should be treated as the best available estimates. These estimates reflect the presently best available knowledge as has been discussed in the previous chapters. Although the calculation procedures may appear to be sophisticated they should only be used when experimental data are not available and measurements can not be performed. If experimental data regarding bioconcentration, biomagnification and food-chain accumulation are available they should be used for exposure assessment purposes rather than the calculated values. Therefore, at all times the use of experimental data is preferable.

7.2 Calculation

The calculation procedures outlined below will distinguish between the following input, internal and output variables:

Input Parameters: (1) Chemical specific parameters -
the 1-octanol-water partition coefficient : K_{OW}
(2) Organism specific parameters -
the organism's weight on a wet weight basis : M (in g)
the organism's lipid content : L_F (in %)
Rate constant for metabolic transformation in organism : k_R (in d^{-1})

(3) Environment specific parameters -
Chemical concentration in the water : C_W (in μg chemical/L water)
Chemical concentration in the food : C_A (in μg chemical/kg food)
The concentration dissolved organic matter in the water C_{DOM} in kg carbon/L of water
The pH of the water

Output Parameters:	C_F	chemical concentration in the fish (in μg chemical/kg organism)
	K_C	the chemical's bioconcentration factor in the organism (only accounts for chemical accumulation from the water)
	K_M	the chemical's biomagnification factor
	K_B	the chemical's bioaccumulation factor in the organism (chemical accumulation from both the water and the food)
	k_1	Rate constant for chemical uptake from the water (in d^{-1})
	k_2	Rate constant for chemical elimination to the water (in d^{-1})
	k_E	Rate constant for chemical elimination by faecal egestion (in d^{-1})

Depending on the amount of available information regarding the characteristics of the chemical, the organism and environmental conditions, estimation of the bioaccumulation parameters can be performed at three levels.

Level I uses the smallest amount of input information. This level only requires chemical specific information regarding the 1-octanol-water partition coefficient. The bioconcentration factor, biomagnification factor and bioaccumulation factor are estimated based on the analogy between the partitioning processes of chemicals between organism lipid tissue and water and 1-octanol and water.

Level II requires knowledge of the chemical's 1-octanol-water partition coefficient and the organism's weight and lipid content. This allows the estimation of the uptake and elimination rate constants of the chemical in the organism of interest. The bioconcentration, biomagnification and bioaccumulation factor are then derived as the ratios of the relevant kinetics rate constants.

Level III is the most accurate method of calculating bioaccumulation parameters. It makes use of information regarding the chemical (i.e. K_{OW}), the organism (i.e. M and L_F) and the environmental conditions. It calculates uptake and elimination rate constants similar to level II. The calculations also provide estimates of the actual concentration of the chemical in the organism based on chemical concentrations in the water and the food to which the organism is exposed. The calculations also take into account the bioavailability of the chemical in the water. When acids are of interest the calculations also make adjustment for chemical dissociation in the water.

When more information about the chemical, the organism and the environmental conditions become available it is possible to make better estimates of the bioaccumulation behaviour of the chemical. Level I calculations therefore provide relatively rough estimates. However, in many cases a rough estimate is sufficient. Level II and III calculations give better estimates but require more initial data.

Level I Calculation

Required input information: K_{OW}
Calculation Output: K_C , K_M , K_B

Calculation Procedure:

$$K_C = 0.05.K_{OW}$$

$$K_M = 10^{0.048.\log KOW - 0.164}$$

$$K_B = 0.05.K_M.K_{OW}$$

Explanation:

Since the available information is limited to the K_{OW} the calculation method incorporates the following assumptions:

(1) An average of 5% is assumed for the lipid content of the organism.
(2) Metabolic transformation in the organism is assumed to be absent.
(3) Chemical excretion into the faeces is assumed to be zero.
(4) 1-Octanol is assumed to simulate the lipid tissue in the organism.
(5) The biomagnification factor is based on an empirical correlation.
(6) The organism is assumed to be in steady state with its environment.

The calculated values for K_C, K_M and K_B should be considered conservative estimates, i.e. they are calculated in a fashion that avoids underestimation.

Level II Calculation

Required input information:
(1) the chemical's K_{OW}
(2) the lipid content of the organism L_F (in %)
(3) the body weight of the organism (wet weight) of the organism M (in grams)
(4) the metabolic rate constant of the chemical in the organism k_R (in d^{-1})

Output information: K_C , K_M , K_B , k_1 , k_2 , k_A , k_E ,

Calculation Procedure:

$$k_1 = 1{,}400/\{(0.71 + [100/K_{OW}]).M^{0.4}\}$$
$$k_2 = 140{,}000/\{L_F.M^{0.4}.(K_{OW} + 100)\}$$
$$k_A = 1/\{2.6.10^{-6}.K_{OW} + 120\}$$
$$k_E = 1/\{7.8.10^{-6}.K_{OW} + 360\}$$

The values for k_1, k_2, k_A and k_E can now be substituted in

$$K_C = k_1/(k_2 + k_E + k_R)$$
$$K_M = k_A/(k_2 + k_E + k_R)$$
$$K_B = k_1/(k_2 + k_E + k_R) + \{(0.05.K_{OW}).k_A/(k_2 + k_E + k_R)\}$$

If information regarding k_R is not available, assume k_R is zero. The values for K_C, K_M and K_B can then be calculated as

$$K_C = k_1/(k_2 + k_E)$$
$$K_M = k_A/(k_2 + k_E)$$
$$K_B = K_C + (0.005.K_{OW}.K_M)$$

Explanation:

If in addition to the chemical's 1-octanol-water partition coefficient the organism's body mass and lipid content are known it is possible to derive a reliable estimate of the rate constants of chemical uptake and depuration. They can then be used to estimate the bioconcentration, biomagnification and bioaccumulation factor. In this calculation procedure the following assumptions are made:

(1) The organism consumes food at a feeding rate of 2% of its own body weight per day.
(2) Chemical in the food is assumed to be in equilibrium with that in the water. The calculations therefore do not take into account food-chain effects.
(3) Faecal egestion rate, expressed as the volume of faeces egested per day, is one third of the ingestion rate, i.e. the volume of food ingested per day.
(4) The lipid content of the food is assumed to be 5%.
(5) 1-Octanol is used to represent the lipids in the organism.
(6) The organism is in steady state with its environment.

Level III Calculation

Required input information:
(1) the chemical's K_{OW}
(2) the lipid content of the organism L_F (in %)
(3) the body weight of the organism (wet weight) of the organism M (in grams)
(4) the metabolic rate constant of the chemical in the organism k_R (in d^{-1})
(5) the chemical concentration in the water C_W (in μg of chemical/L of water)
(6) the chemical concentration in the food C_A in (μg of chemical/kg of food)
(7) The concentration sorbing organic matter in the water C_{SOR} in kg carbon/L of water, i.e. typically 10^{-5} to 10^{-6}. C_{SOR} is the group $M_S f$ where M_S is the suspended solids concentration and f is the mass fraction organic carbon.

If these calculations are to be performed for acids two other properties should be known, i.e.

(8) the dissociation constant of the chemical, i.e. pK_a
(9) the pH of the water

Output information: K_C , K_M , K_B , k_1 , k_2 , k_A , k_E , C_F

Calculation Procedure:

Calculation of chemical uptake and depuration constants by substituting values for K_{OW} and M in the following equations

$$k_1 = 1{,}400/\{(1 + [100/K_{OW}]).M^{0.4}\}$$
$$k_2 = 1{,}400/\{L_F.M^{0.4}.(K_{OW} + 100)$$
$$k_A = 1/\{2.6.10^{-6}.K_{OW} + 120\}$$
$$k_E = 1/\{7.8.10^{-6}.K_{OW} + 360\}$$

K_C, K_M and K_B can now be calculated by substituting the values for k_1, k_2, k_A and k_E, as derived above, in:

$$K_C = k_1/(k_2 + k_E + k_R)$$
$$K_M = k_A/(k_2 + k_E + k_R)$$
$$K_B = K_C + (C_A.K_M/C_W)$$

If information regarding k_R is not available, k_R will be assumed to be zero. The values for K_C, K_M and K_B can then be calculated as

$$K_C = k_1/(k_2 + k_E)$$
$$K_M = k_A/(k_2 + k_E)$$
$$K_B = K_C + (C_A.K_M/C_W)$$

The available solute fraction ASF in the water is calculated as

$$ASF = 1/\{1 + (C_{SOR}.K_{OW})\}$$

where $C_{SOR} = M_sf$

If no value for C_{SOR} is available , a typical value of 10^{-6} kg/L should be used resulting in

$$\mathrm{ASF} = 1/\{1 + (10^{-6}.K_{OW})\}$$

If ASF as calculated here proves to be less than 0.75 the solids concentration effect should be included and ASF recalculated using Eq 2.12:

$$K_P = fK_{OW}/(1 + 0.7 \text{ x } 10^{-6}\, fM_S\, K_{OW})$$

and

$$\mathrm{ASF} = 1/(1 + K_P M_S)$$

The concentration in the organism C_F can then be calculated as

$$C_F = C_W.\mathrm{ASF}.K_B$$

In case the chemical of interest is an acid and C_W represents the total chemical concentration in the water (i.e. ionized and nonionized forms of the acid), C_F can be estimated as

$$C_F = \mathrm{ASF}.K_B.C_W/(10^{pH-pKa} + 1)$$

Explanation :

(1) The calculation of the ASF is based on the assumption that the organic carbon sorption coefficient K_P equals K_{OW}, and that C_{SOR} represents the concentration of sorbing organic matter in the water column. In contrast to other assumptions made in the calculations above, the scientific evidence supporting the assumption that K_{OW} is a satisfactory surrogate for K_P is limited. As discussed earlier it is often difficult to measure the concentration of organic matter in the water, and values for C_{SOR} for Ontario waters are very rare.

Examples

Two examples are presented below illustrating the three levels of calculation procedures outlined above. The first example involves the chemical trichlorobenzene, which has a K_{OW} of 10,000. The second example involves the chemical mirex, which is a very hydrophobic chemical with a K_{OW} of 32,000,000.

Level I

Input: K_{OW} of trichlorobenzene is 10,000

Output: K_C of trichlorobenzene is $0.05*10{,}000 = 500$
log K_M of trichlorobenzene is:
$0.048*\log(10{,}000) - 0.164 = 0.028$
K_M is thus $10^{0.028} = 1.07$
K_B of trichlorobenzene is $0.05*1.07*10{,}000 = 535$

Input: K_{OW} of mirex is 32,000,000

Output: K_C of mirex is $0.05*32{,}000{,}000 = 1{,}600{,}000$
log K_M of mirex is:
$0.048*\log(32{,}000{,}000) - 0.164 = 0.196$
K_M of mirex is thus $10^{0.196} = 1.57$
K_B of mirex is $0.05*1.57*32{,}000{,}000 = 2{,}512{,}000$

Level II

In the level II calculation we will make estimates for the K_C, K_M and K_B of trichlorobenzene and mirex in a particular species of fish. In this example we will assume a 5 gram fathead minnow, with a lipid content of 6%. Trichlorobenzene is metabolized by the fish. The rate constant for metabolic transformation k_R is 0.6 d^{-1}. Mirex is not metabolized by the fish, the rate constant k_R thus being zero.

Input: K_{OW} of trichlorobenzene is 10,000
L_F of the fathead minnow is 6%
M of the fathead minnow is 5 grams
k_R is 0.6 d^{-1}

Model: $k_1 = 1{,}400/\{(1 + [100/K_{OW}])*M^{0.4}\}$
$k_2 = 140{,}000/\{L_F*M^{0.4}*(K_{OW} + 100)\}$
$k_A = 1/\{2.6.10^{-6}*K_{OW} + 120\}$
$k_E = 1/\{7.8.10^{-6}*K_{OW} + 360\}$

Output: $k_1 = 1{,}400/\{(1 + [100/10{,}000])*5^{04}\} = 728\ d^{-1}$
$k_2 = 140{,}000/\{6*5^{04}*(10{,}000 + 100)\} = 1.2\ d^{-1}$
$k_A = 1/\{2.6.10^{-6}*10{,}000 + 120\} = 0.0083\ d^{-1}$
$k_E = 1/\{7.8.10^{-6}*10{,}000 + 360\} = 0.0028\ d^{-1}$

K_C, K_M and K_B can now be calculated by substituting the values for k_1, k_2, k_A and k_E, as derived above, in:

$$K_C = k_1/(k_2 + k_E)$$
$$K_M = k_A/(k_2 + k_E)$$
$$K_B = K_C + (0.05*K_{OW}*K_M)$$

This results in:

$$K_C = 728/(1.2 + 0.0028 + 0.6) = 404$$
$$K_M = 0.0083/(1.2 + 0.0028 + 0.6) = 0.0046$$
$$K_B = 404 + (0.05*10{,}000*0.0046) = 406$$

The calculations illustrate that of the total chemical eliminated by the fish 67% is to the water (via the gills), 33% is by metabolic transformation and only 0.16% is by faecal egestion. The chemical concentration in the fish is 406 fold higher than the concentration in the water. Biomagnification and food-chain accumulation are insignificant as reflected by the very low value for K_M. The bioaccumulation factor is thus approximately equal to the bioconcentration factor. The chemical concentration in the fish is predominantly the result of chemical uptake from the water: 404*100/406, i.e. 99.5% of the TCB body burden in the fish is absorbed from the water and 0.5% is taken up from the food.

For mirex the calculations are performed as follows:

Input: K_{OW} of mirex is 32,000,000
L_F of the fathead minnow is 6%
M of the fathead minnow is 5 grams
k_R is 0 d^{-1}

Output:

$$k_1 = 1{,}400/\{(1 + [100/32{,}000{,}000])*5^{0.4}\} = 735\ d^{-1}$$
$$k_2 = 140{,}000/\{6*5^{0.4}*(32{,}000{,}000 + 100)\} = 0.00038\ d^{-1}$$
$$k_A = 1/\{2.6.10^{-6}*32{,}000{,}000 + 120\} = 0.0049\ d^{-1}$$
$$k_E = 1/\{7.8.10^{-6}*32{,}000{,}000 + 360\} = 0.0016\ d^{-1}$$

K_C, K_M and K_B can now be calculated by substituting the values for k_1, k_2, k_A and k_E, as derived above, in :

$$K_C = 735/(0.00038 + 0.0016 + 0) = 370{,}000$$
$$K_M = 0.0049/(0.00038 + 0.0016 + 0) = 2.5$$
$$K_B = 370{,}00 + (0.05*32{,}000{,}000*2.5) = 4{,}370{,}000$$

The calculations illustrate that of the total amount of mirex eliminated by the fish 81% is to the faeces and 19% is to the water. The chemical concentration in the fish is 4,370,000 times higher than the concentration in the water. Biomagnification and thus food-chain accumulation are very important as reflected by the high value of 2.5 for K_M, which is close to the maximum value of 3. Uptake of mirex from food can thus be a significant exposure route. The relative importance of mirex uptake from food and water depends on the mirex concentrations in the water and the food of the fish. Level II calculations assume a chemical equilibrium of mirex between the water and the food. Under this condition the fish will absorb 8.5% mirex from the water (i.e. 370,000*100/4,370,000) and 91.5% mirex from its food source.

Level III

The level III calculations estimate the chemical concentrations in the organism based on information regarding the chemical, the organism and specific environmental conditions. In this example we will again perform calculations for mirex and trichlorobenzene in a 5g fathead minnow with a 6% lipid content. This time, however, we will assume a typical Great Lakes aquatic ecosystem with water concentrations of 80 pg/L and 30 pg/L, and food concentrations of 0.02 μg/Kg and 8 μg/Kg for TCB and mirex respectively. The concentration dissolved organic carbon in the water is 10^{-6}.

Input: K_{OW} of trichlorobenzene is 10,000
L_F of the fathead minnow is 6%
M of the fathead minnow is 5 grams
k_R is 0.6 d^{-1}
C_W is 80.10^{-6} $\mu g/L$
C_A is 0.02 $\mu g/kg$
C_{SOR} is 10^{-6} kg/L

Model:
$k_1 = 1{,}400/\{(1 + [100/K_{OW}])*M0.4\}$
$k_2 = 140{,}000/\{L_F*M^{0.4}*(K_{OW} + 100)\}$
$k_A = 1/\{2.6.10^{-6}*K_{OW} + 120\}$
$k_E = 1/\{7.8.10^{-6}*K_{OW} + 360\}$

Output:
$k_1 = 1{,}400/\{(1 + [100/10{,}000])*5^{0.4}\} = 728\ d^{-1}$
$k_2 = 140{,}000/\{6*5^{0.4}*(10{,}000 + 100)\} = 1.2\ d^{-1}$
$k_A = 1/\{2.6.10^{-6}*10{,}000 + 120\} = 0.0083\ d^{-1}$
$k_E = 1/\{7.8.10^{-6}*10{,}000 + 360\} = 0.0028\ d^{-1}$

K_C, K_M and K_B can now be calculated by substituting the values for k_1, k_2, k_A and k_E, as derived above, in:

$$K_C = k_1/k_2 + k_E + k_R)$$
$$K_M = k_A/(k_2 + k_E + k_R)$$
$$K_B = K_C + (C_A*K_M/C_W)$$

This results in:

$$K_C = 728/(1.2 + 0.0028 + 0.6) = 404$$
$$K_M = 0.0083/(1.2 + 0.0028 + 0.6) = 0.0046$$
$$K_B = 404 + (0.02*0.0046/80.10^{-6}) = 405.2$$

The available solute fraction ASF in the water is calculated as

$$ASF = 1/\{1 + (C_{SOR}.K_{OW)\}}$$

i.e. $$ASF = 1/\{1 + (10^{-6}*10{,}000)\} = 0.99$$

There is no need to include a solids concentration "correction".

The concentration in the organism C_F can then be calculated as

$$C_F = 80.10^{-6}*0.99*405.2 = 0.032\ \mu g/kg$$

For mirex the calculations are performed as follows:

Input:
K_{OW} of mirex is 32,000,000
L_F of the fathead minnow is 6%
M of the fathead minnow is 5 grams
k_R is 0 d^{-1}
C_W is 30.10^{-6} $\mu g/L$
C_A is 8.0 $\mu g/kg$
C_{SOR} is 10^{-6} kg/L

Output:
$k_1 = 1{,}400/\{(1 + [100/32{,}000{,}000])*5^{0.4}\} = 735\ d^{-1}$
$k_2 = 140{,}000/\{6*5^{0.4}*(32{,}000{,}000 + 100)\} = 0.00038\ d^{-1}$
$k_A = 1/\{2.6.10^{-6}*32{,}000{,}000 + 120\} = 0.0049\ d^{-1}$
$k_E = 1/\{7.8.10^{-6}*32{,}000{,}000 + 360\} = 0.0016\ d^{-1}$

K_C, K_M and K_B can now be calculated by substituting the values for k_1, k_2, k_A and k_E, as derived above, in :

$$K_C = 735/(0.00038 + 0.0016 + 0) = 370{,}000$$
$$K_M = 0.0049/(0.00038 + 0.0016 + 0) = 2.5$$
$$K_B = 370{,}000 + (8*2.5/30.10^{-6}) = 1{,}040{,}000$$

The available solute fraction ASF in the water is calculated as

$$ASF = 1/\{1 + (10^{-6}*32{,}000{,}000)\} = 0.03$$

A solids concentration convention should be applied. Assuming f of 1.0 and M_S of 10^{-6}

$$K_P = 32.10^6/\{1 + (0.7*10^{-6}*32.10^6)\} = 1.37.10^6$$

$$ASF = 1/(1 + 1.37.10^6*10^{-6}) = 0.42$$

The concentration in the organism C_F can then be calculated as

$$C_F = 30.10^{-6}*0.42*1{,}040{,}000 = 13.1\ \mu g/kg$$

Assuming a chemical with properties similar to trichlororbenzene, but which is an acid with a pK_a of 5, and water with a pH of 6 then C_F can be estimated as

$$C_F = 80.10^{-6}*(1/(10^{6-5} + 1)*0.99*405.2 = 0.0029\ \mu g/kg$$

8. BIOCONCENTRATION TESTS AND DATA HANDLING

8.1 Introduction

To determine the bioaccumulation tendency of substances various bioaccumulation tests can be performed. Most common is the bioconcentration test.

Bioconcentration experiments in the laboratory are usually performed by exposing a species of interest to an aqueous solution of the test chemical for a certain length of time. A variety of experimental procedures have been used to measure bioconcentration factors and bioconcentration rate constants. In this section we will briefly summarize the existing techniques.

8.2 Preparation of Aqueous Solutions

Aqueous solutions used in bioconcentration experiments are usually prepared with either carrier solvents or with a generator column technique.

When carrier solvents are used to make aqueous solutions a known amount of the test chemical is dissolved in a carrier solvent and then applied to the water. The amount of chemical that is dissolved in the carrier solvent should not exceed the maximum amount of chemical that can be dissolved in the volume of water, to which the chemical is eventually applied. This amount of chemical can be calculated from the chemical's aqueous solubility and the volume of water in the exposure system.

The carrier solvent should meet the following requirements:

(1) it should dissolve the amount of chemical that will be applied to the water.
(2) it should mix with water.

(3) it should disappear quickly from the water, usually by evaporation, after it has been applied.
(4) it should not be toxic to the test organisms at the volume applied.
(5) it should not intervene with the uptake and bioaccumulation of the test chemicals.

Carrier solvents which have often been used are dichloromethane, acetone and ethanol, but other solvents may be suitable as well.

Solutions of the test chemical in the carrier solvent are usually applied to the water by slow injection onto the surface of the water. Then the water is stirred for a considerable time or left to equilibrate, after which the test organisms are added. This method is often used because of its simplicity and speed. The disadvantage of this procedure is that the test chemical often tends to precipitate after it has been applied to the water. This is especially the case for very hydrophobic chemicals, which have very low aqueous solubilities. Precipitation of the chemical is due to the rapid evaporation of the carrier solvent, after it has been applied. This results in a quick release of the test chemical to the water, which leads to precipitation in the form of flocking or micro-crystal formation. Stirring helps to dissolve the chemical in the water. It is therefore recommended that a period of 2 to 3 days of stirring is maintained before the test organisms are added. This method is considered to be unsuitable for preparing aqueous solutions of very hydrophobic chemicals (i.e. with aqueous solubilities smaller than 100 ppb).

To keep the aqueous concentrations constant throughout the exposure period some authors deliver their test chemical solution continuously to the water by using (peristaltic) pumps. This procedure introduces carrier solvent in the water. This may affect the physiological and metabolic functions of the test organism during the bioconcentration experiments as well as alter the form of the test chemical in solution.

The generator column technique is often used to measure the aqueous solubilities of hydrophobic chemicals. This technique was first described by Veith and Compton and optimised by May et al. (1978a,1978b) to allow reliable measurements of the aqueous solubility of very hydrophobic chemicals. Various modifications of this method have been used to measure aqueous solubilities and to make aqueous solutions for bioconcentration experiments (Bruggeman et al. 1981, Gobas et al. 1989, Opperhuizen et al. 1985).

A known amount of the test chemical, usually approximately 1% of the weight of the carrier material, is dissolved in an organic solvent such as n-pentane, n-hexane, acetone, dichloromethane or toluene. Then a carrier material, usually glass-beads or Chromosorb, is added to this solution. This solution is stirred for approximately 15 minutes after which the solvent is evaporated completely while stirring continuously. This procedure ensures that the chemical is coated onto the glass beads or Chromosorb. The coated carrier material is then packed in a column, through which the water is passed. A glass filter or simply glass wool can be used to prevent coated carrier material from entering the exposure system. The contact area between carrier material and water and the flow rate, with which the water is passed through this column, determine the chemical concentration in the water. The water concentrations will therefore depend on the characteristics and geometry of the system. However, when applied correctly the chemical concentration in the water will not exceed the aqueous solubility of the chemical. Specific conditions regarding the flow rate and geometry requirements for aqueous solubility measurements can be found in May et al. (1978a and 1978b) and Shiu et al. (1988). When this method is used to prepare aqueous solutions for bioconcentration experiments it is usually not necessary to adopt these conditions.

Bruggeman et al.(1981), Opperhuizen et al. (1985), Gobas et al. (1988) and others have used the generator column technique with great success in bioconcentration experiments. These authors prepared saturated aqueous solutions for hydrophobic and very hydrophobic chemicals using a continuous flow system (Figure 8.1), where the water was continuously pumped through the column until the chemical concentration in the water reached a constant level, which corresponded with the chemical's aqueous solubility. Solutions below the aqueous solubility can be prepared by pumping water through this system for a shorter duration.

The generator column technique is a reliable technique to prepare aqueous solutions of organic chemicals and is superior over the "carrier solvent method".

8.3 The Static Bioconcentration Test

The static system is the simplest method to measure bioconcentration. According to this method an aqueous solution of the test chemical(s) is prepared, after which the test organisms are added. The water is not replaced or recirculated. If bioconcentration occurs the chemical concentration in the organism increases with time and the concentration in the water decreases as a result of chemical transfer from the water to the organism until steady state has been achieved. Figure 8.1

illustrates the variation in the concentration in the organism and water with time in a typical static test when the bioconcentration process follows the two-compartment (organism-water) model with first order rate constants.

Several authors have successfully used the static system to measure the bioconcentration factor and bioconcentration rate constants. For example Banerjee et al. (1984) measured the bioconcentration factor and uptake and elimination rate constants of some halobenzenes in three fish species and found values that were approximately similar to those derived by Koneman and Van Leeuwen (1980) and Veith et al. (1980) who used flow-through systems. Wang et al.(1982), Geyer et al. (1984) and Mailhot (1987), used the static system to measure the bioconcentration factors in various species of algae.

Several methods can be applied to deduce the bioconcentration factor and rate constants from the observed concentrations in the organism and water, i.e.

(i) The bioconcentration factor can be determined from the ratio of the chemical concentrations in the organism and water (C_F/C_W), after changes in C_F and C_W can no longer be detected (i.e. steady state has been reached, Figure 8.1). This method has been applied by Wang et al. (1982) and Geyer et al. (1984).

(ii) Assuming that the bioconcentration process follows the two-compartment model with first order rate constants the following equation can be used to determine the rate constants k_1 and k_2 from the decrease in the concentration in the water with time (Banerjee et al., 1984):

$$C_{W,}t/C_{W,0} = \{k_2+(k_1.F.\exp-[(k_1.F+k_2).t])\}/(k_1.F+k_2) \qquad (8.1)$$

where C_{W},t is the concentration in the water at time t, $C_{W,0}$ is the initial concentration in the water and F is the mass of the fish per unit mass of water. The rate constants k_1 and k_2 are then fitted to produce the best agreement with the observed concentrations in the water. An iterative computer program such as STATIC developed by Kim (1970) can be used for the data fitting. The bioconcentration factor can then be calculated as k_1/k_2.

It should be noted that methods (i) and (ii) obtain reliable values for k_1 and k_2 only when there is no loss of chemical from the static system through processes such as evaporation, degradation,

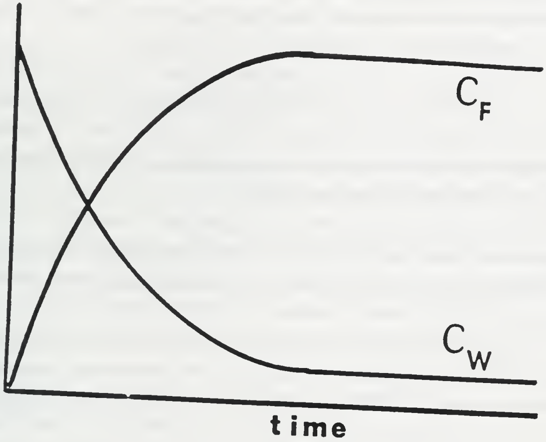


Figure 8.1

The organism (C_F) and water (C_W) concentration as a function of time in a static bioconcentration experiment with no loss of chemical from the system.

metabolic transformation or adsorption to the container or tank. This should therefore be checked before methods (i) or (ii) are used by establishing a mass balance of the chemical in the system. A mass balance is a budget on the total amount of chemical in the water and organism. It calculates the total mass of chemical in the system X_t from the total volumes of water and organism and the concentration in water and organisms at every time t as

$$X_t = V_W.C_W + V_F.C_F \tag{8.2}$$

If X_t is constant at all times throughout the experiment methods (i) and (ii) will give reliable values for the bioconcentration factor and rate constants.

In many bioconcentration experiments there is net loss of chemical from the system. In that case the following procedure can be used to deduce the bioconcentration parameters (Gobas et al., 1988)

(iii) First, the time function of the chemical concentration in the water should be established. Frequently, the chemical concentration in the water drops during the experiment. This drop in C_W over time can often be described by the following time functions:

$$C_W = C_{W,0}.\exp(-A.t) \tag{8.3}$$

or

$$\log C_W = B.t^2 + C.t + D \tag{8.4}$$

where A,B,C,D are constants, which can be fitted to the observed concentrations in the water by regression techniques. If equation 8.3 is used to establish the time function of the concentration in the water it is helpful to convert the observed C_W to the natural logarithm. The constant A can then be determined by performing a linear regression on the ln C_W vs. t data i.e.,

$$\ln C_W = -A.t + \ln C_{W,0} \tag{8.5}$$

where A follows from the slope of the regression. When equation 8.4 is used to fit the C_W-t data multiple linear regression of the log C_W data versus time will obtain the values for the constants B,C and D.

After the time function of the concentration in the water has been established a numerical integration is applied to fit the observed chemical concentrations in the organism. This numerical integration involves the summation of increments in organism concentration dC_F over time periods of dt, which can be calculated from

$$dC_F = (k_1.C_W - k_2.C_F).dt \tag{8.6}$$

where C_W at each exposure time t follows from either equation 8.3 or equation 8.4 or any other reliable fit of the concentration in the water and C_F is the sum of all previous dC_F. The time steps dt should be sufficiently small (rule of thumb: $dt = (1/k_2)/30)$). Values for the rate constants k_1 and k_2 are then selected to produce the best agreement between fitted and observed concentrations in the fish. An iterative computer program, which repeatedly chooses values for k_1 and k_2 and tests the quality of the fit, can be used for this purpose. The best fit of the data is the one which results in the smallest sum of squared differences between the estimated and observed chemical concentrations in the fish. It is preferable to measure the elimination rate constant k_2 independently in a depuration experiments. If the elimination rate constant is known only k_1 has to be fitted by equation 8.6. The bioconcentration factor can then determined as k_1/k_2.

This method can also be used for the analysis of data in other bioconcentration systems. It is particularly useful when the concentration in the water varies during the experiment. Other advantages are that the derived bioconcentration factor is independent of the actual length of the exposure period.

Instead of performing a numerical integration it is also possible to substitute the C_W-time function in equation 8.6 and then integrate this equation to derive the time function for C_F. This function can then be used to fit the observed concentrations in the organism for example by non-linear regression.

The advantages of the static bioconcentration test are its simplicity, its relatively short duration and low costs. It also allows the derivation of the bioconcentration factor and rate constants from water concentration data alone. The main drawbacks are the limited applicability of this technique and the lack of agreement with exposure conditions in the environment. The static test gives reliable bioconcentration data for low K_{OW} chemicals, which reach steady-state relatively quickly (within 3 to 5 days) and do not significantly metabolize and evaporate from the system. This technique is generally unsuitable for very hydrophobic chemicals with a high K_{OW} and for large organisms. The continuing decrease of the chemical concentration in the water throughout the experiment makes a direct comparison with the more constant exposure conditions in the environment difficult.

8.4 Continuous Flow System

Several authors have used a continuous flow system to determine the bioconcentration factor and kinetics of organic chemicals including chemicals with very high K_{ow}. Bruggeman et al. (1980, 1983, 1984) used a continuous flow system to measure the bioconcentration kinetics of PCBs, polydimethylsiloxanes and a series of super-lipophilic chemicals in gold fish and guppies. Opperhuizen et al. (1985) and Gobas et al. (1989) slightly modified this system and used it to measure the bioconcentration of a wide variety of chemicals such as polychlorinated naphthalenes, PCBs, chlorobenzenes, bromobenzenes, chlorodibenzo-p-dioxins and bromobiphenyls in the guppy.

The continuous flow system consists of a water contamination system, usually a generator-column, through which the water in the exposure tank is circulated continuously. A sedimentation tank and a glass filter are sometimes included to remove particulate matter, introduced by the organism, from the exposure tank.

Before the start of the uptake experiment the water is recirculated (typical flow rate: 50 L/d) for some time to build up the desired chemical concentration in the water. If the water is recirculated for a long enough period the aqueous solutions will reach its saturation limit. Then the organisms are added to the exposure system to start the uptake experiment. Usually, water recirculation is continued for a certain period and then stopped, after which the system is left to equilibrate. During this equilibration phase chemical concentration in the water decreases and the concentration in the organism further increases with time until steady-state has been achieved. Water circulation is usually stopped to prevent the build-up of toxic levels of the contaminant in the organism and to shorten the time to reach steady-state.

The bioconcentration factor and rate constants can be derived from the observed concentrations in organism and water in various manners:

(i) If steady state has been achieved during the experiment the bioconcentration factor K_C can be determined from the chemical concentrations in the fish and the water i.e. C_F/C_W at steady state. When the elimination rate constant k_2 has been determined independently in a depuration experiment, k_1 can be calculated as $k_2.K_C$.

(ii) If steady-state is not reached at the end of the exposure period a numerical integration procedure, which was discussed earlier for static systems, can be used. This method first establishes the time function of the concentration in the water and then performs a (numerical) integration to fit the observed C_F data, resulting in values for k_1 and k_2. It is preferable that the elimination rate constant k_2 is determined in a separate experiment.

The main advantage of the continuous flow system over the static system is the better control of the concentration in the water during the experiments. This is especially important for bioconcentration measurements of very hydrophobic, high K_{OW} chemicals. Since the concentration in the water drops during the equilibration period exposure conditions usually do not match the more constant contaminant levels in the environment. The continuous flow systems are generally easier and cheaper to run and are therefore often preferred over the more complicated flow-through system.

8.5 Flow-Through Systems

The most frequently used bioconcentration test is using a flow-through system to contaminate the water. The main characteristic of the flow-through system is that the chemical concentration in the exposure tank is held constant by continuously replacing the aqueous solution by a newly prepared aqueous solution. This is usually achieved by pumping uncontaminated water through a generator column at a constant rate into the exposure tank and then out of the system (Muir et al. 1986). Other reported flow-through systems make use of carrier solvents, which are applied with a peristaltic pump, to prepare the aqueous solutions (Oliver and Niimi 1983, Branson et al. 1985).

Several modifications have been successfully used by Branson et al. (1975, 1985), Veith et al. (1979), Oliver and Niimi (1983), McKim et al. (1986), Muir et al. (1986) and others. For a detailed description of the flow-through systems used by these authors the reader may want to refer to the original authors.

When the chemical concentration in the water is constant throughout the exposure period the chemical concentration in the organism can usually be described by the following relationship with time:

$$C_F = C_W.(k_1/k_2).\{1 - \exp(-k_2.t)\} \tag{8.7}$$

The rate constants k_1 and k_2 can be estimated from the data using non-linear regression or specific data-handling packages such as BIOFAC (Neely) or Acutti-59 (Butte and Blum 1984).

If steady state has been achieved within the duration of the experiment and the elimination rate constant has been determined independently in a depuration experiment, K_C can be calculated as C_F/C_W and k_1 as $K_C.k_2$.

If the concentration in the water varies with time the numerical integration procedure discussed earlier can be used to derive the bioconcentration parameters.

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